Sequencing alignment

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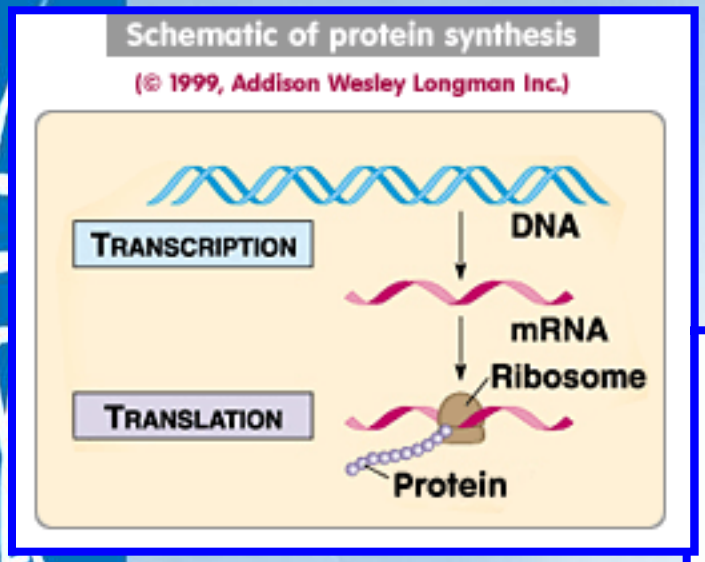
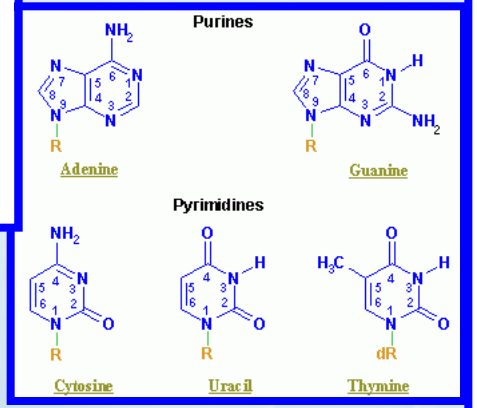
Why perform a multiple sequence alignment?

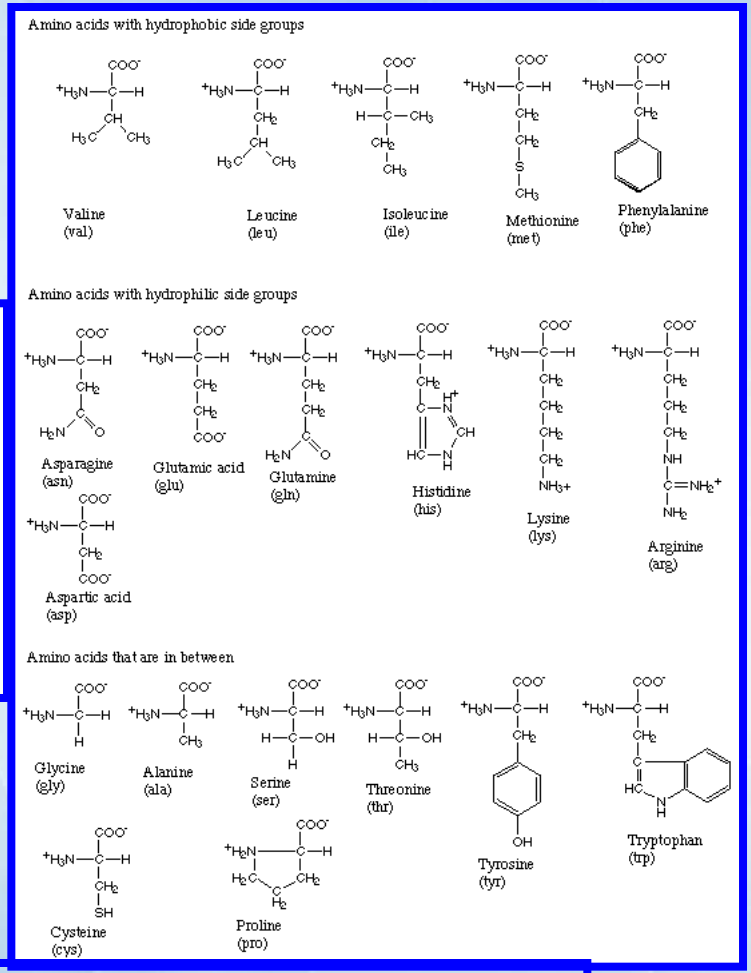
MSAs are at the heart of comparative genomics studies which seek to study evolutionary histories, functional and structural aspects of sequences, and to understand phenotypic differences between species

The Building Blocks…





ATGC



VLMFNQEDHKRCSTPYW

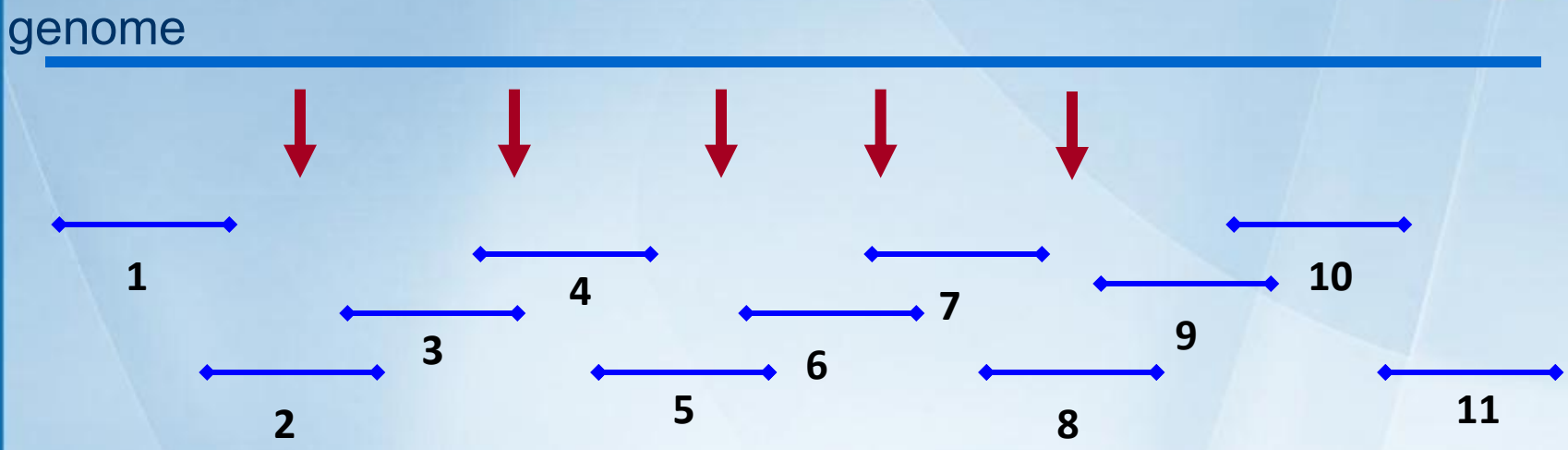
```
MVNLTSDEKTAVLALWNKVDVEDCGGEALGRLLVVYPWTQRFFE...
||  ||   ||||| |||  ||  ||    ||||||||||||||||||
MVHLTPEEKTAVNALWGKVNVDAVGGEALGRLLVVYPWTQRFFE...
```

Alignment: Comparing two (pairwise) or more (multiple) sequences. Searching for a series of identical or similar characters in the sequences.

Why Align Sequences?

- Discover functional, structural, and evolutionary information
- Similar Sequences may have similar function
  - Gene Regulation
  - Biochemical Function
  - Similar Structure
- Identify primers and probes to search for homologous sequences in other organisms
- Crucial for genome sequencing

Genome Sequencing Strategy



1. Obtain a large collection of BAC clones
2. Map them onto the genome (Physical Mapping)
3. Select a minimum tiling path
4. Sequence each clone in the path with shotgun
5. Assemble
6. Put everything together

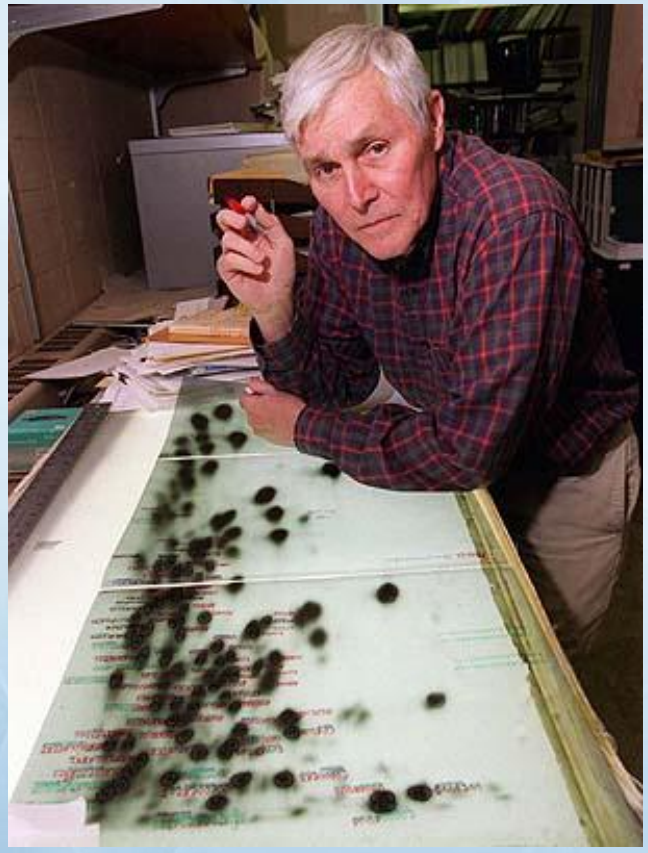
Why Align Sequences?

- Homology
  - Similar sequences may have a common ancestor

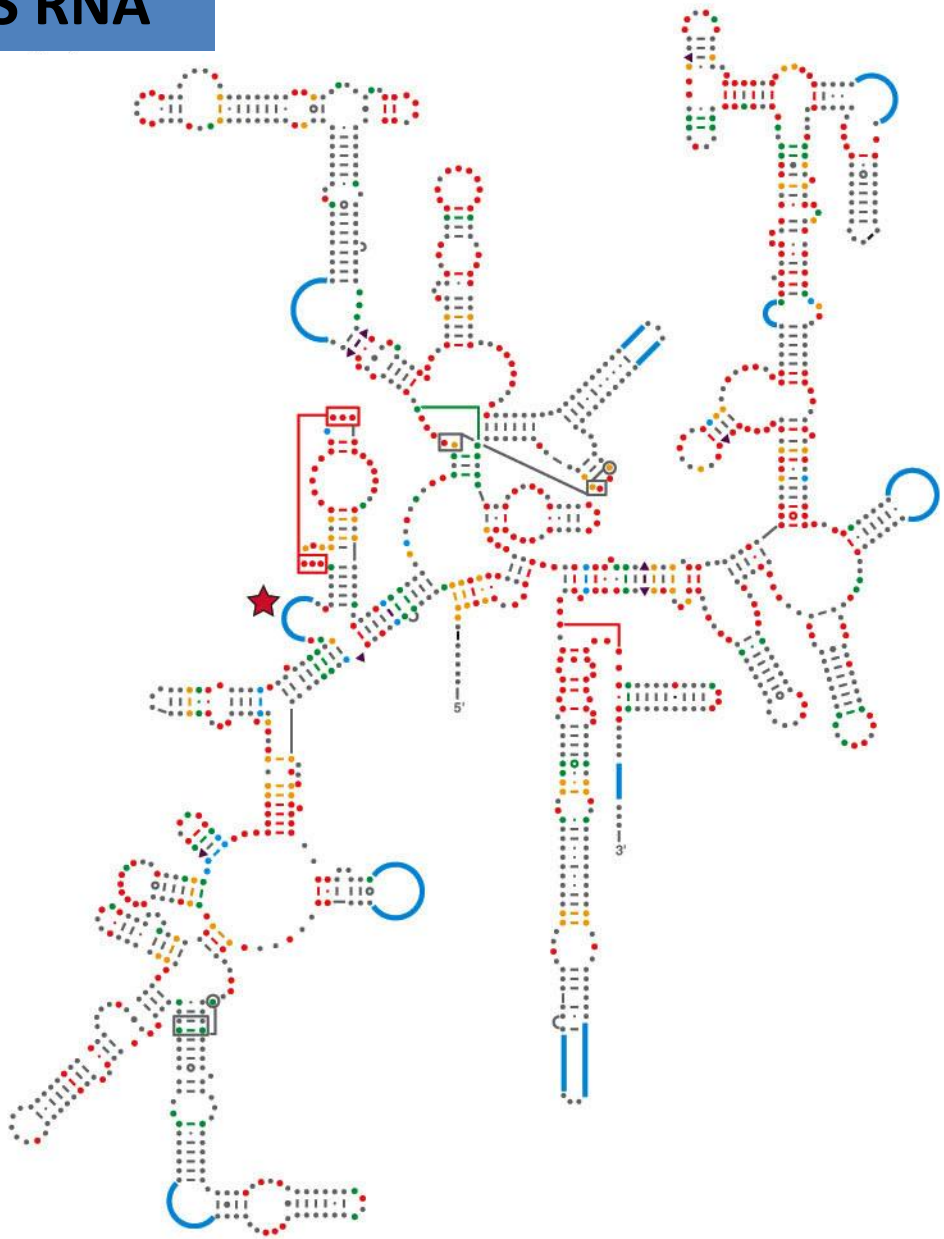
- In 1990 Carl Woese and colleagues proposed the Tree of Life, using a molecular phylogenetic approach.

- It is based on sequencing rRNA (16S and 18S), which all organisms share.

- All organisms were separated into three domains: *Bacteria*, *Archaea*, *Eukarya*.

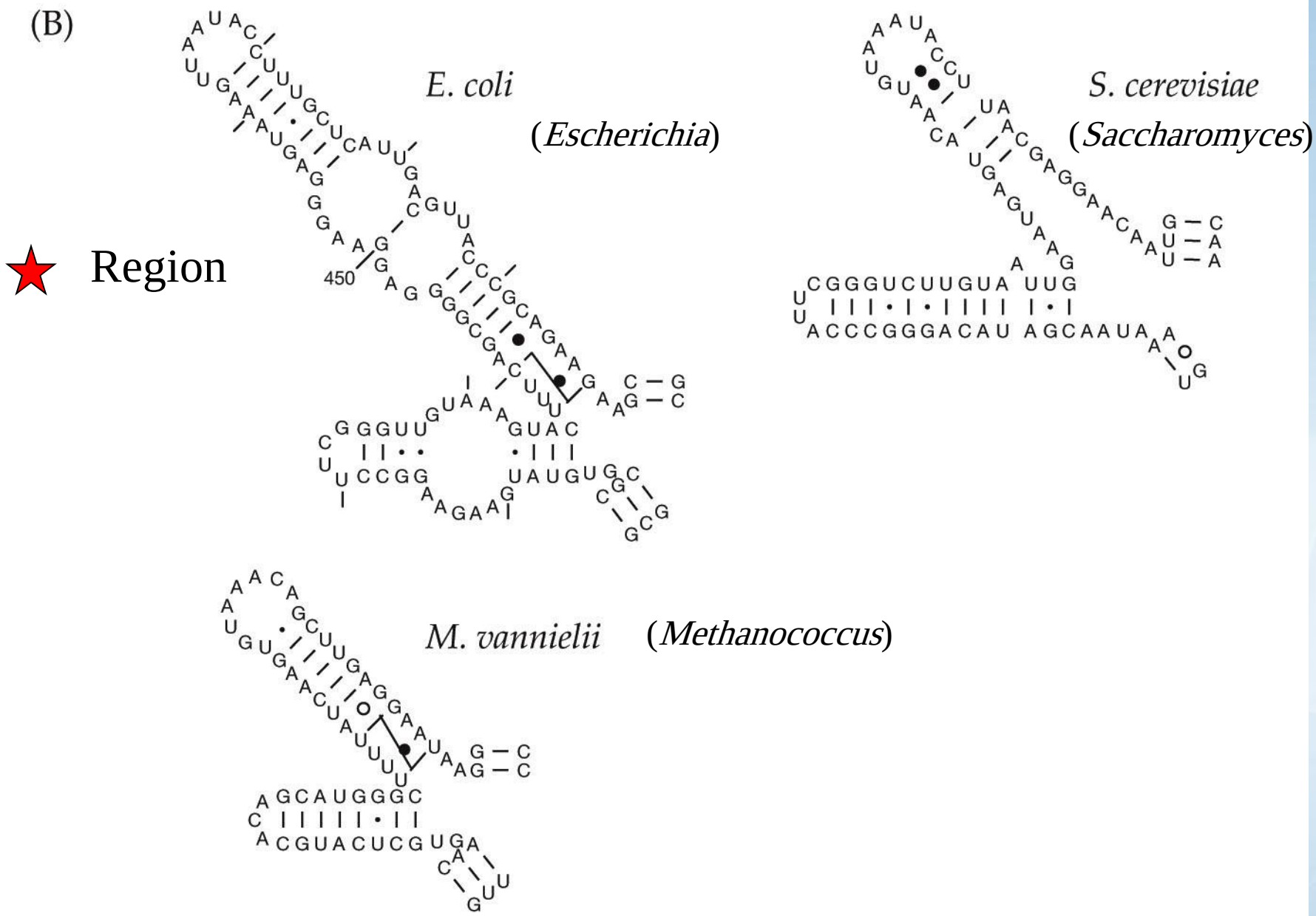


16S RNA

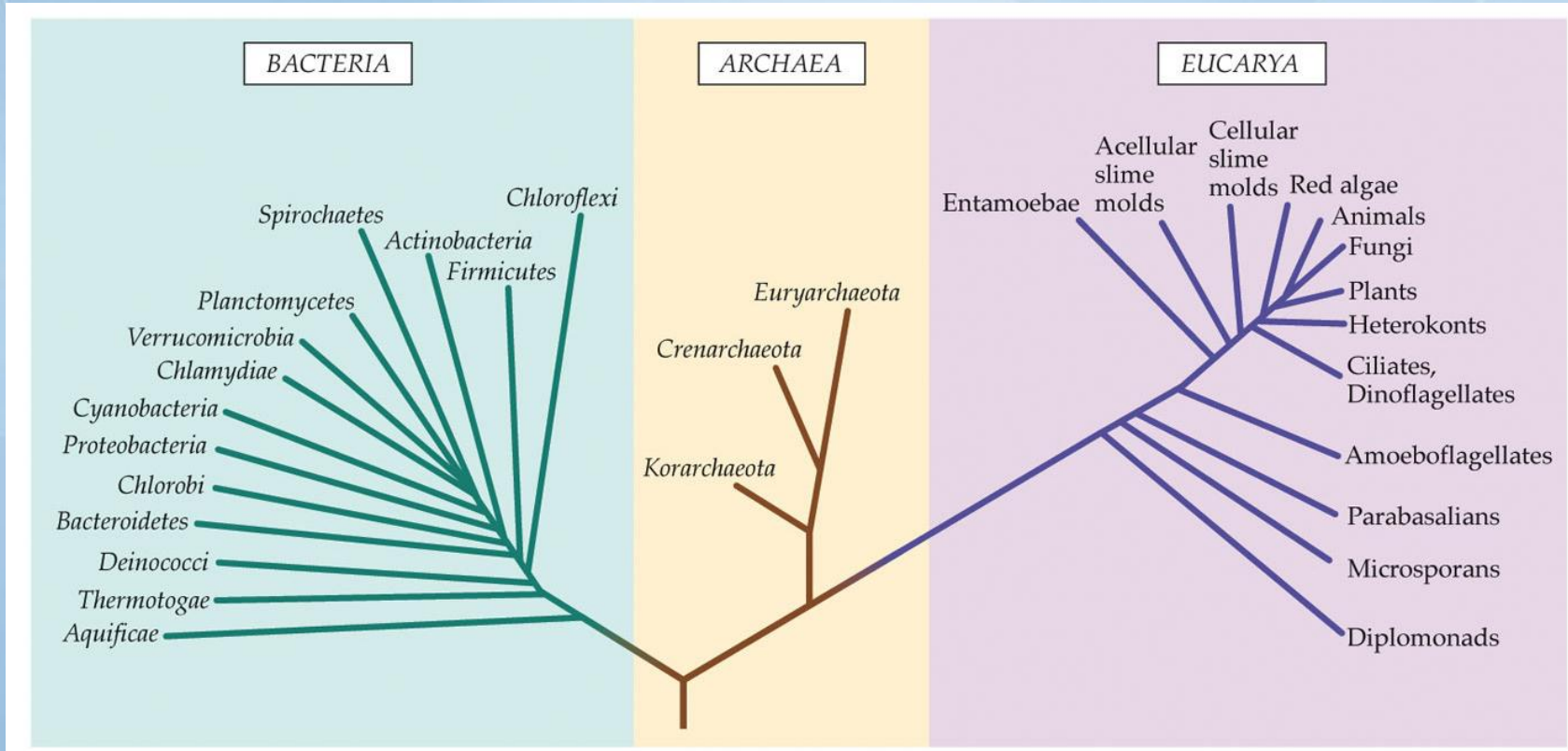


- Identical in 98% or more of all organisms
- Conserved only in the *Bacteria*
- Conserved only in the *Archaea*
- Conserved only in the *Eukarya*
- ▲ Conserved within each domain, variable among domains
- Regions that vary structurally among domains

***Microbial Life 2e*, Figure 17.3 (Part 1)**



Microbial Life 2e, Figure 17.3 (Part 2)


BACTERIA
ARCHAEA
EUCARYA
Chloroflexi
Spirochaetes
Actinobacteria
Firmicutes
Planctomycetes
Verrucomicrobia
Chlamydiae
Cyanobacteria
Proteobacteria
Chlorobi
Bacteroidetes
Deinococci
Thermotogae
Aquificae
Euryarchaeota
Crenarchaeota
Korarchaeota
Entamoebae
Acellular slime molds
Cellular slime molds
Red algae
Animals
Fungi
Plants
Heterokonts
Ciliates, Dinoflagellates
Amoeboflagellates
Parabasalians
Microsporans
Diplomonads

Evolutionary changes in sequences

Three types of nucleotide changes:

1. ***Substitution*** – a replacement of one (or more) sequence characters by another: **AAGA → AACA**
2. ***Insertion*** - an insertion of one (or more) sequence characters: **AAG A**
3. ***Deletion*** – a deletion of one (or more) sequence characters: **A A GA**

Insertion + Deletion → Indel

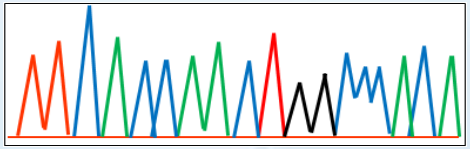
How DNA Sequence Data is Compared

Obtain Samples:
Blood , Saliva, Hair
Follicles, Feathers, Scales

Genetic Data

Extract DNA from Cells

Sequence DNA



...TTCACCAACAGGCCCACA...

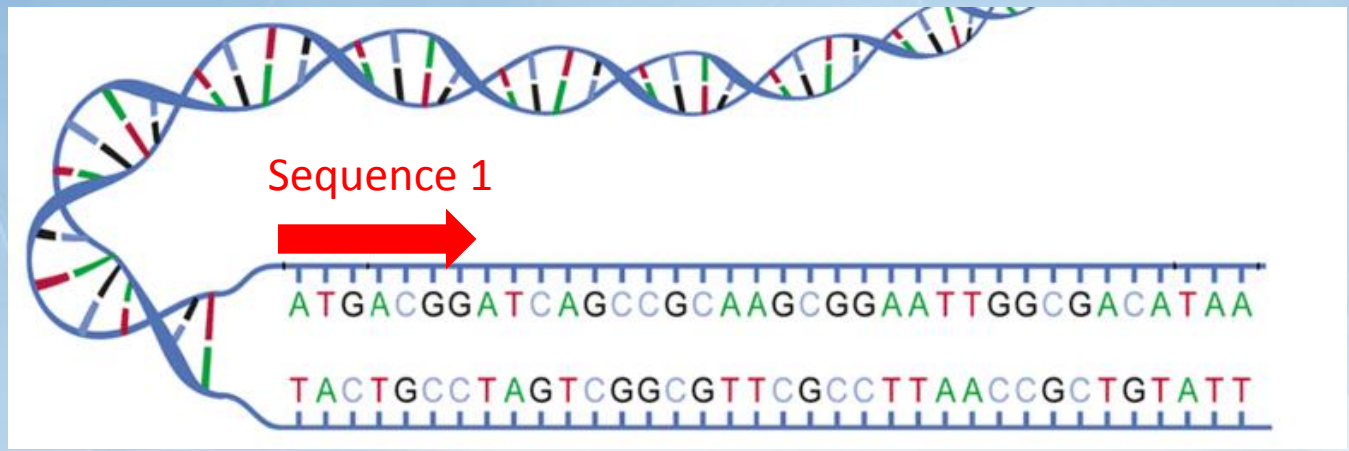
Compare DNA Sequences to One Another

TTCAACAACAGGCCCAC
TTCACCAACAGGCCCAC
TTCATCAACAGGCCCAC

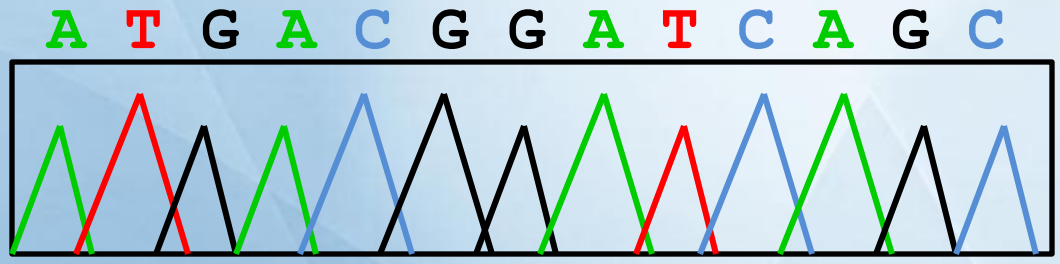
GOALS:

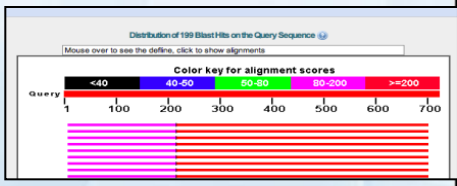
- Identify the organism from which the DNA was obtained.
- Compare DNA sequences to each other.

Sequence Both Strands of DNA



Sequence :

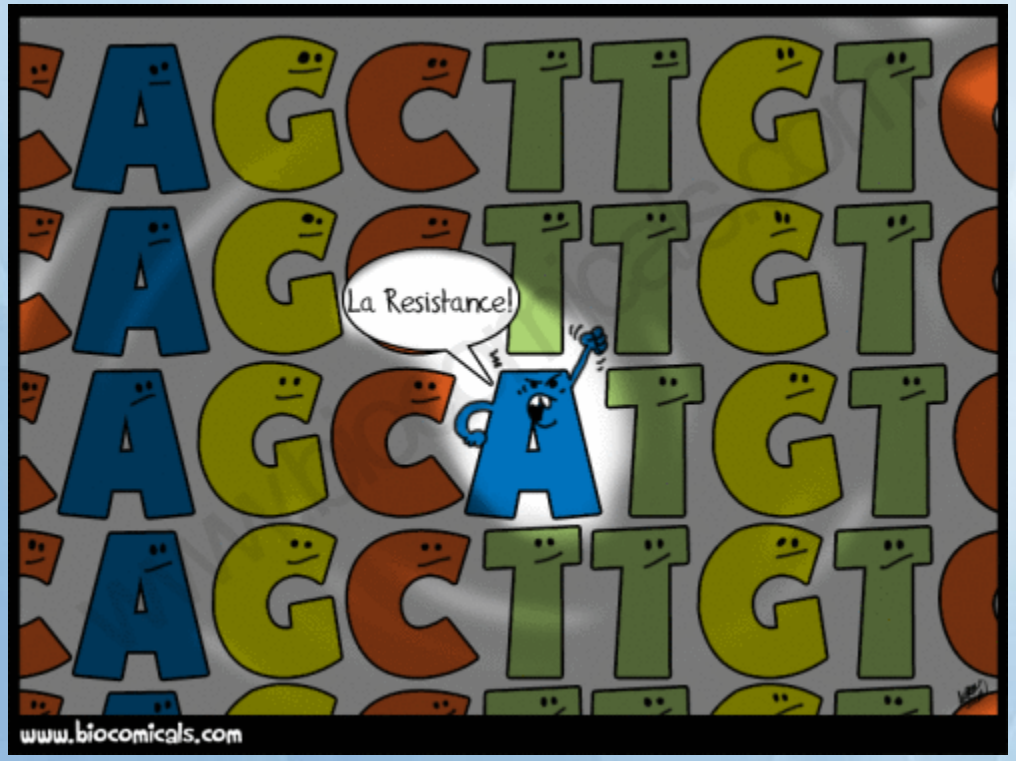




Bioinformatics tools like **BLAST** can be used to compare the sequences from Different individuals.

Comparing two sequences

```
MVNLTSDEKTAVLALWNKVDVEDCGGE
||  ||   |||||  |||  ||  ||    ||
MVHLTPEEKTAVNALWGKVNVDAVGGE
```



Multiple sequence alignment

```
Seq1 VTISCTGSSSNIGAG-NHVKWYQQLPG
Seq2 VTISCTGTSSNIGS--ITVNWYQQLPG
Seq3 LRLSCSSSGFIFSS--YAMYWVRQAPG
Seq4 LSLTCTVSGTSFDD--YYSTWVRQPPG
Seq5 PEVTCVVVDVSHEDPQVKFNWYVDG--
Seq6 ATLVCLISDFYPGA--VTVAWKADS--
Seq7 AALGCLVKDYFPEP--VTVSWNSG---
Seq8 VSLTCLVKGFYPSD--IAVEWWSNG--
```

Similar to pairwise alignment BUT ***n*** sequences are aligned instead of just **2**

Each row represents an individual sequence

Each column represents the ‘same’ position

Multiple sequence alignment

```
Seq1  VTISCTGSSSNIGAG-NHVKWYQQLPG
Seq2  VTISCTGTSSNIGS--ITVNWYQQLPG
Seq3  LRLSCSSSGFIFSS--YAMYWVRQAPG
Seq4  LSLTCTVSGTSFDD--YYSTWVRQPPG
Seq5  PEVTCVVVDVSHEDPQVKFNWYVDG--
Seq6  ATLVCLISDFYPGA--VTVAWKADS--
Seq7  AALGCLVKDYFPEP--VTVSWNSG---
Seq8  VSLTCLVKGFYPSD--IAVEWWSNG--
```

Multiple Sequence Alignment: Approaches

- Local alignment – finds regions of high similarity in parts of the sequences

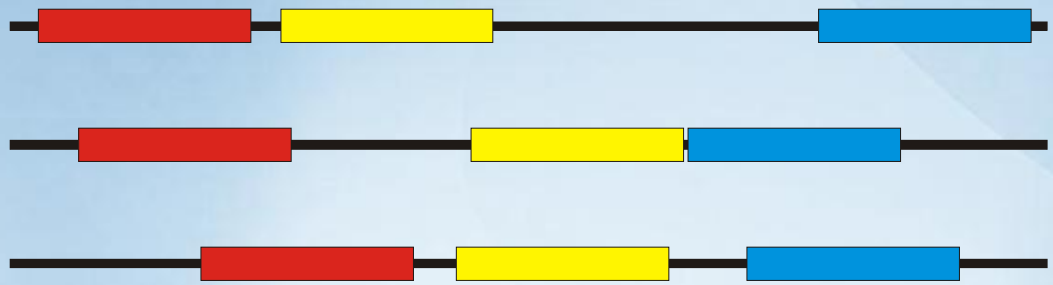
```
ADLGAVFALCDRYFQ
||||      ||||  |
ADLGRTQN CDRYYQ
```

- Global alignment – finds the best alignment across the entire two sequences

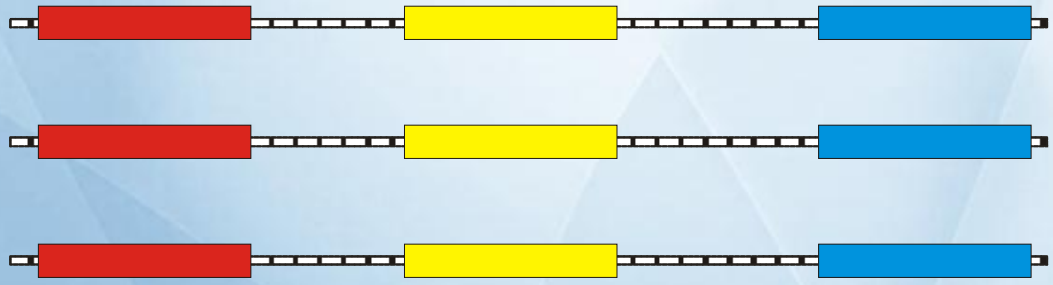
```
ADLGAVFALCDRYFQ
||||      ||||  |
ADLGRTQN-CDRYYQ
```

How to optimize alignment algorithms?

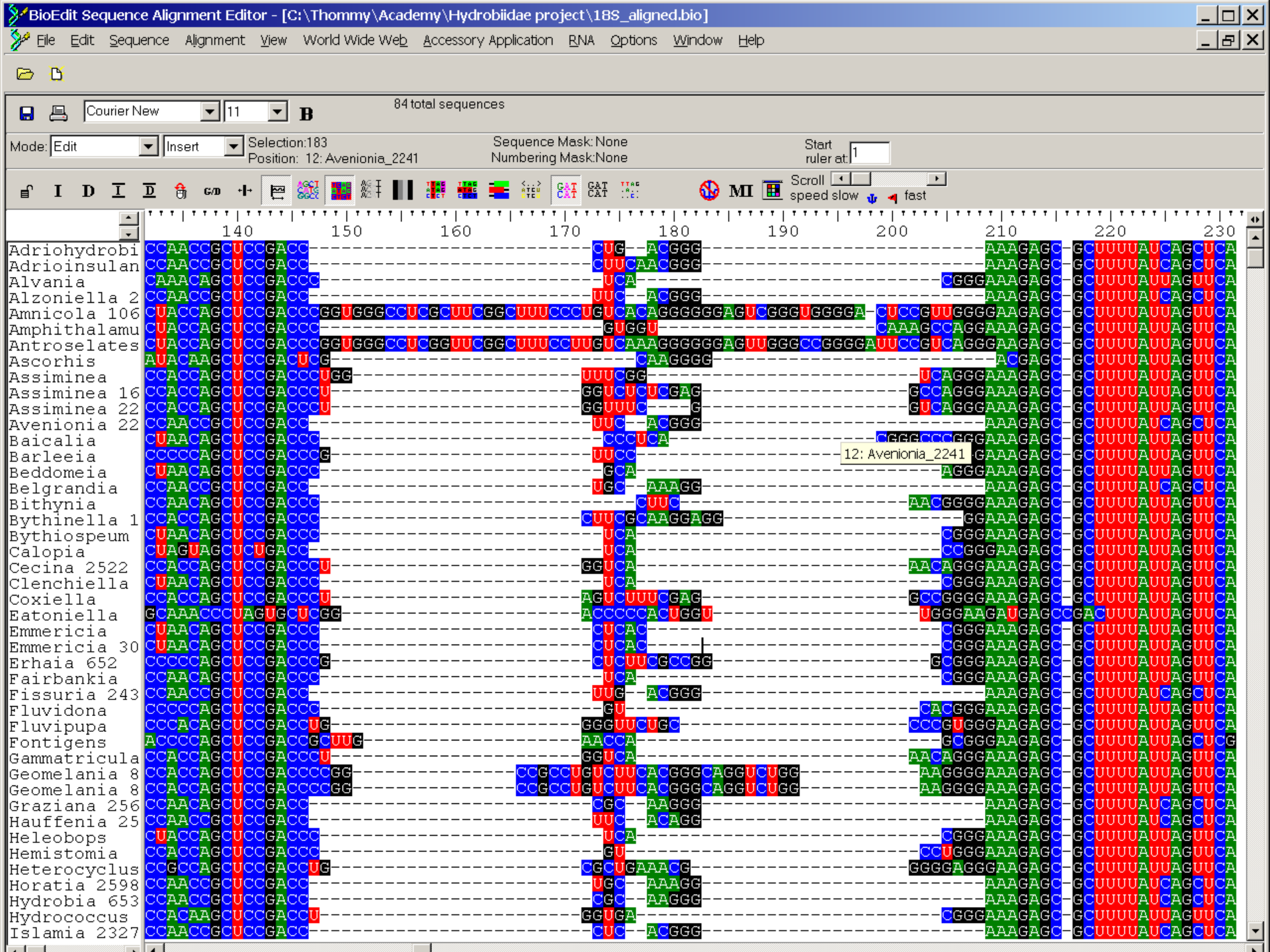
- Sequences often contain highly conserved regions



These regions can be used for an initial alignment



By analyzing a number of small, independent fragments, the algorithmic complexity can be drastically reduced!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File Edit Sequence Alignment View World Wide Web Accessory Application RNA Options Window Help
Courier New
11
84 total sequences
Mode: Edit
Insert
Selection:183
Position: 12: Avenionia_2241
Sequence Mask: None
Numbering Mask:None
Start ruler at: 1
Scroll speed slow fast
140 150 160 170 180 190 200 210 220 230
12: Avenionia_2241
Adriohydrobi
Adrioinsulan
Alvania
Alzoniella 2
Amnicola 106
Amphithalamu
Antroselates
Ascorhis
Assiminea
Assiminea 16
Assiminea 22
Avenionia 22
Baicalia
Barleeia
Beddomeia
Belgrandia
Bithynia
Bythinella 1
Bythiospeum
Calopia
Cecina 2522
Clenchiella
Coxiella
Eatoniella
Emmericia
Emmericia 30
Erhaia 652
Fairbankia
Fissuria 243
Fluvidona
Fluvipupa
Fontigens
Gammatricula
Geomelania 8
Geomelania 8
Graziana 256
Hauffenia 25
Heleobops
Hemistomia
Heterocyclus
Horatia 2598
Hydrobia 653
Hydrococcus
Islamia 2327

Choosing an alignment:

- Many different alignments between two sequences are possible:

```
AAGCTGAATTCGAA
AGGCTCATTTCTGA
```

```
AAGCGAAATTCGAAC
A-G-GAA-CTCGAAC
```

```
AAGCGAAATTCGAAC
AGG---AACTCGAAC
```

How do we determine which is the best alignment?

Assessing the significance of an alignment score

True

AAGCTGAATTCGAA AGGCTCATTTCTGA	AAGCTGAATTC-GAA AGGCTCATTTCTGA- 28.0

Random

AGATCAGTAGACTA GAGTAGCTATCTCT	AGATCAGTAGACTA----- ----GAGTAG-CTATCTCT 26.0
.	
.	
CGATAGATAGCATA GCATGTCATGATTC	CGATAGATAGCATA--------- ---------GCATGTCATGATTC 16.0

"Optimal" vs. "correct" alignment

For a given group of sequences, there is no single "correct" alignment, only an alignment that is "optimal" according to some set of calculations

This is partly due to:

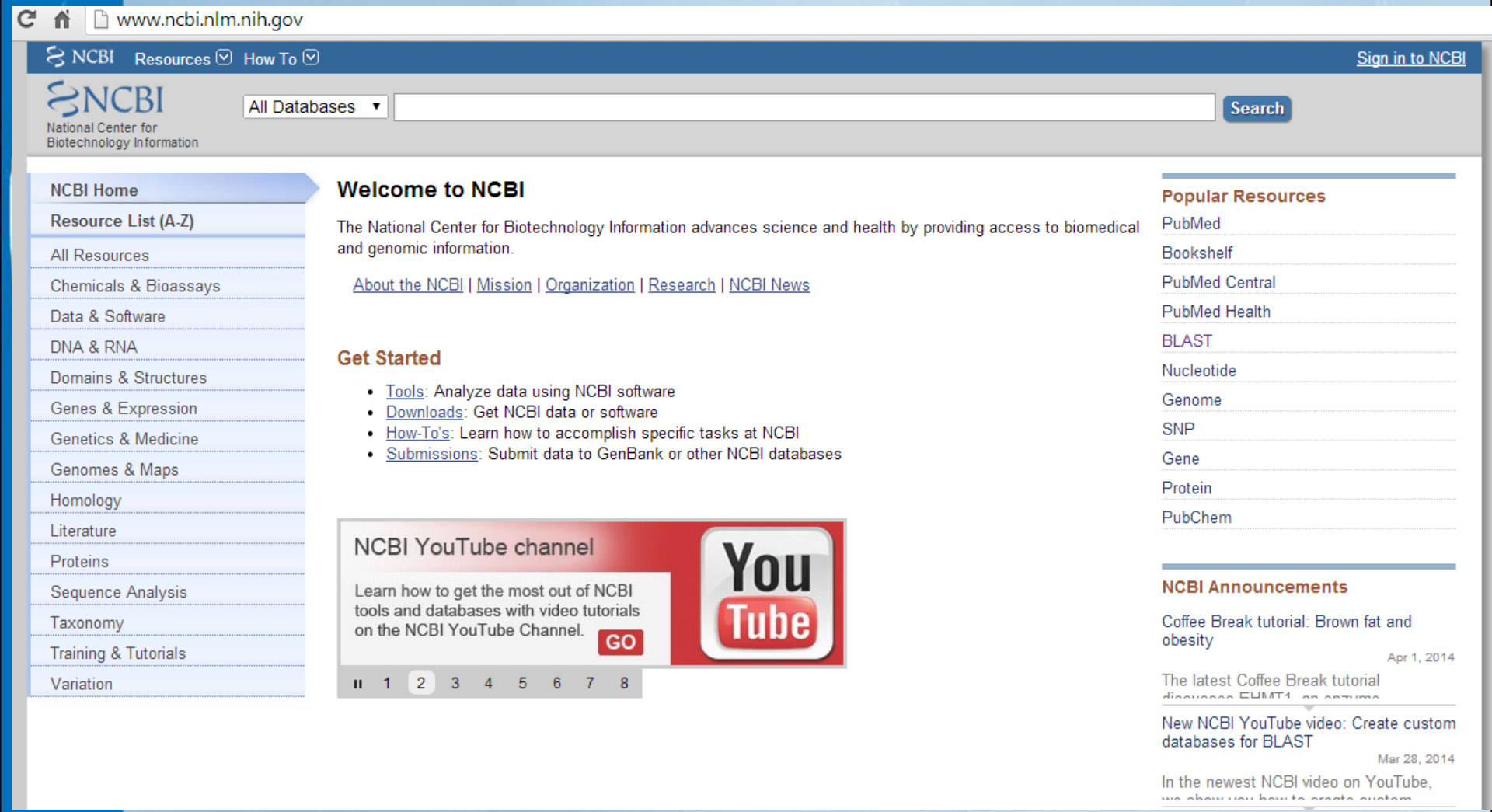
- the complexity of the problem,
- limitations of the scoring systems used,
- our limited understanding of life and evolution

Determining what alignment is best for a given set of sequences is really up to the judgment of the investigator

Success of the alignment will depend on the similarity of the sequences. If sequence variation is great it will be very difficult to find an optimal alignment

Web servers for pairwise alignment

NCBI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Search
NCBI Home
Resource List (A-Z)
All Resources
Chemicals & Bioassays
Data & Software
DNA & RNA
Domains & Structures
Genes & Expression
Genetics & Medicine
Genomes & Maps
Homology
Literature
Proteins
Sequence Analysis
Taxonomy
Training & Tutorials
Variation
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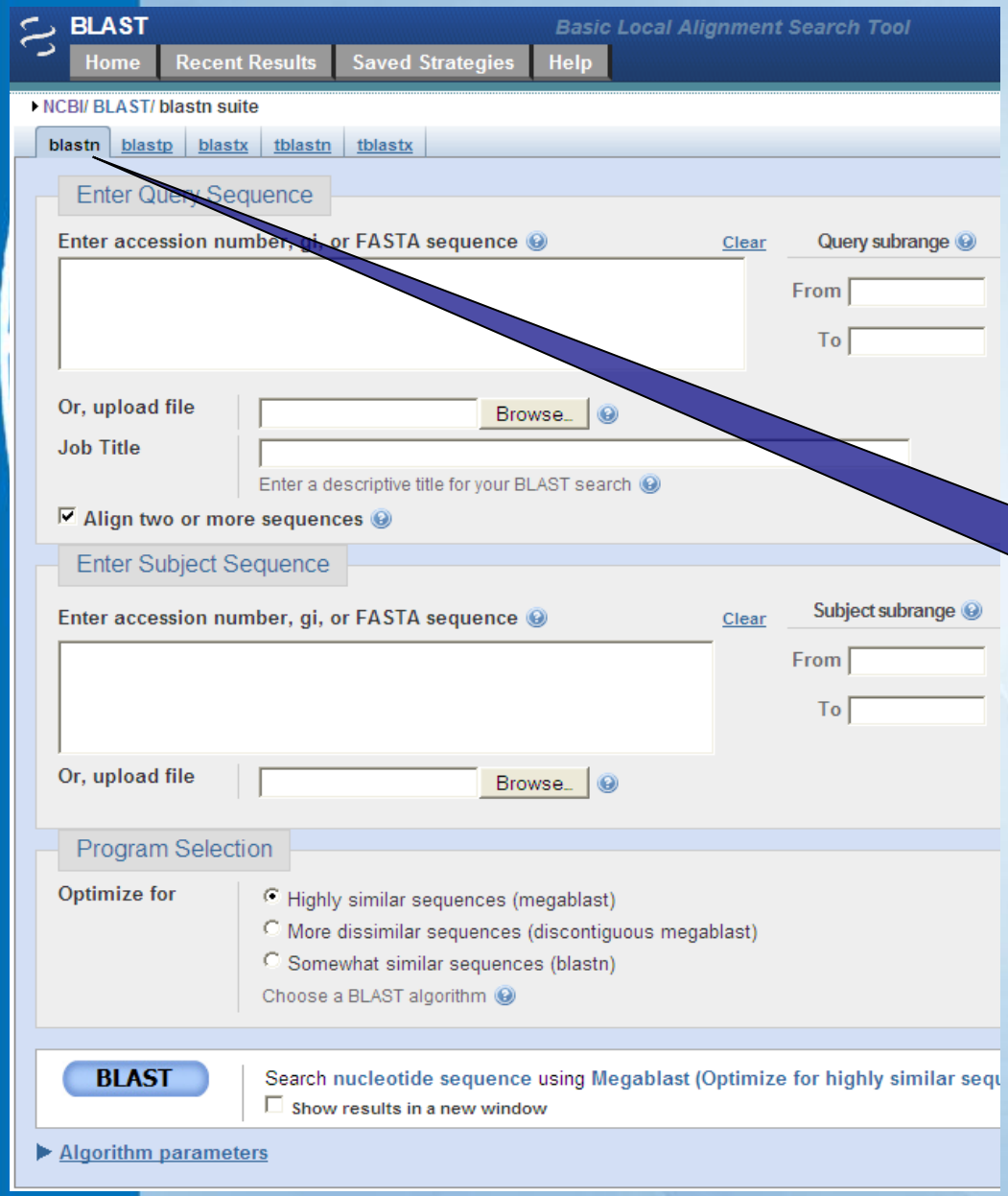
Genome
SNP
Gene
Protein
PubChem
NCBI Announcements
Coffee Break tutorial: Brown fat and obesity
Apr 1, 2014
The latest Coffee Break tutorial
New NCBI YouTube video: Create custom databases for BLAST
Mar 28, 2014
In the newest NCBI video on YouTube,

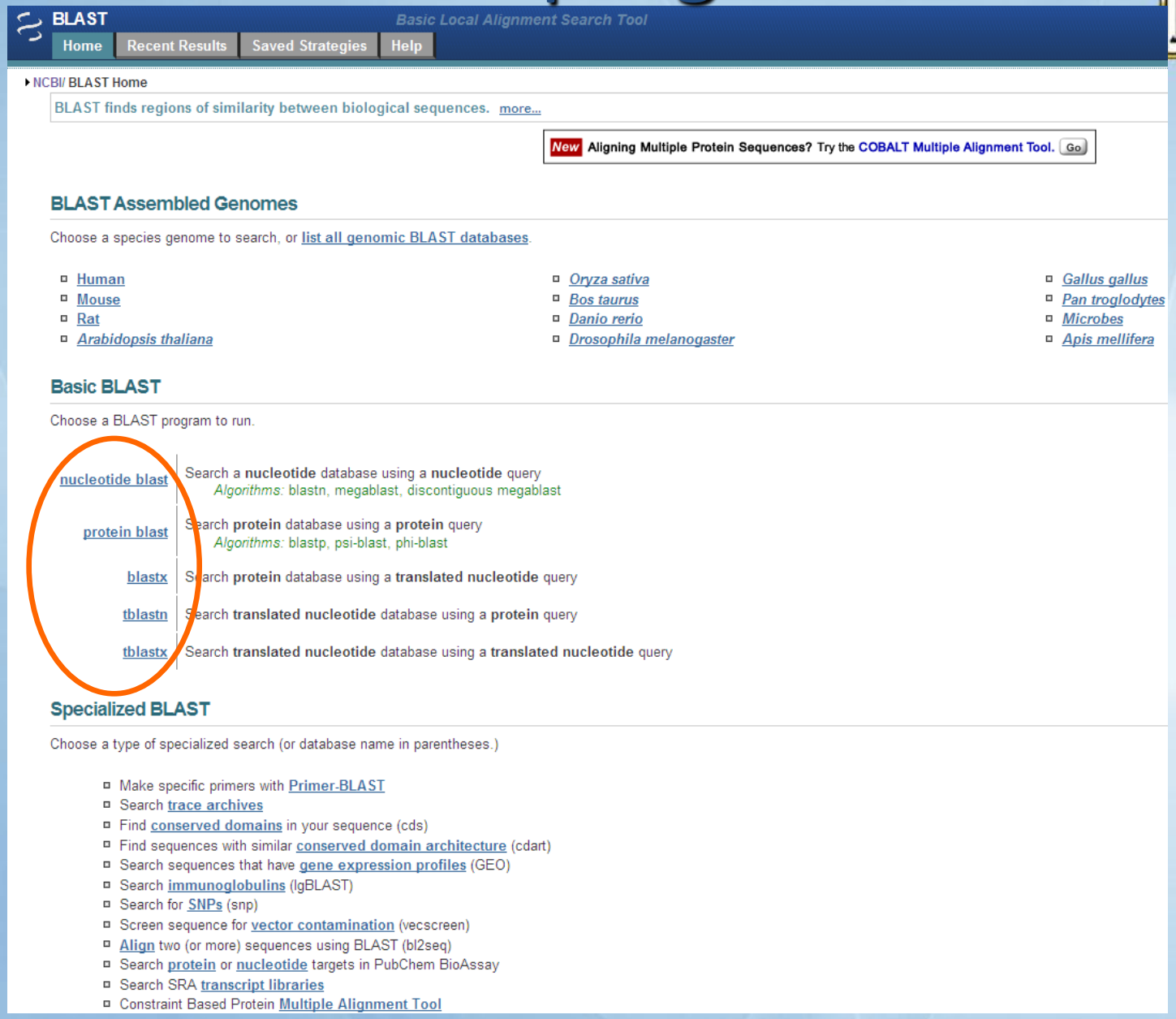
Basic Local Alignment Search Tool



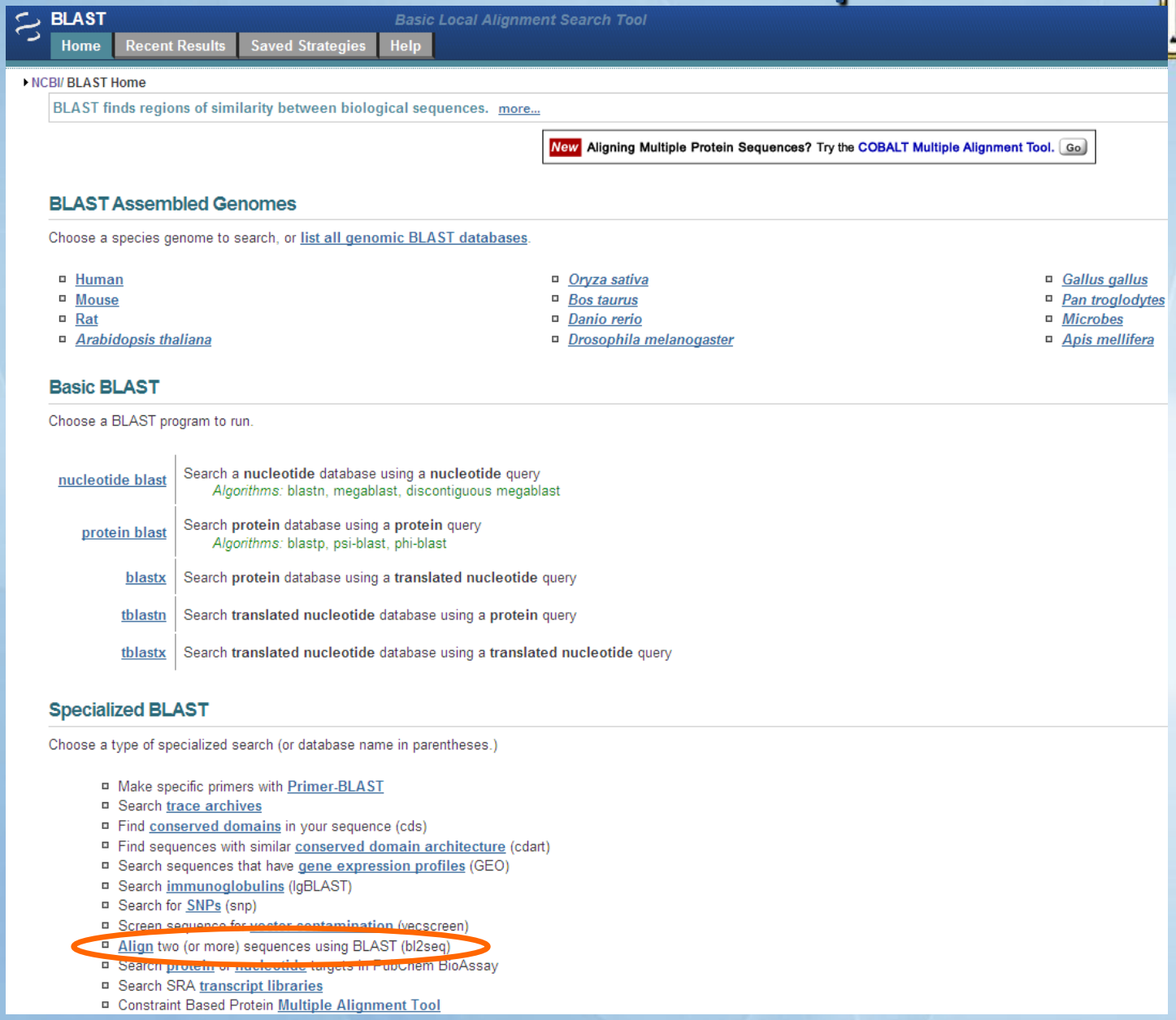
blastn – nucleotide

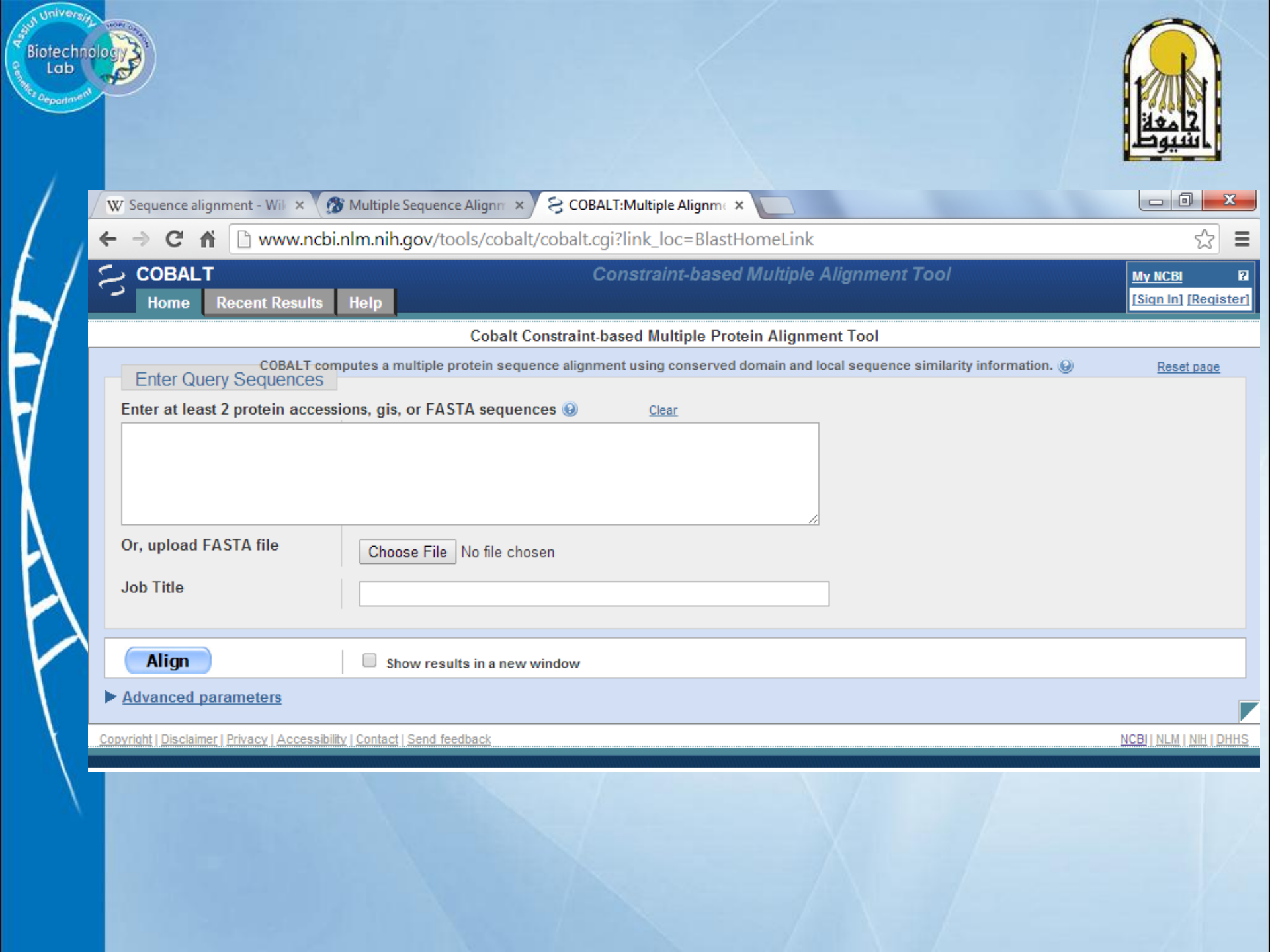
blastp – protein

BLAST – programs



BLAST – bl2seq




Sequence alignment - Wi
Multiple Sequence Alignm
COBALT:Multiple Alignm
www.ncbi.nlm.nih.gov/tools/cobalt/cobalt.cgi?link_loc=BlastHomeLink
COBALT
Constraint-based Multiple Alignment Tool
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Home
Recent Results
Help
Cobalt Constraint-based Multiple Protein Alignment Tool
COBALT computes a multiple protein sequence alignment using conserved domain and local sequence similarity information.
Reset page
Enter Query Sequences
Enter at least 2 protein accessions, gis, or FASTA sequences
Clear
Or, upload FASTA file
Choose File
No file chosen
Job Title
Align
Show results in a new window
Advanced parameters
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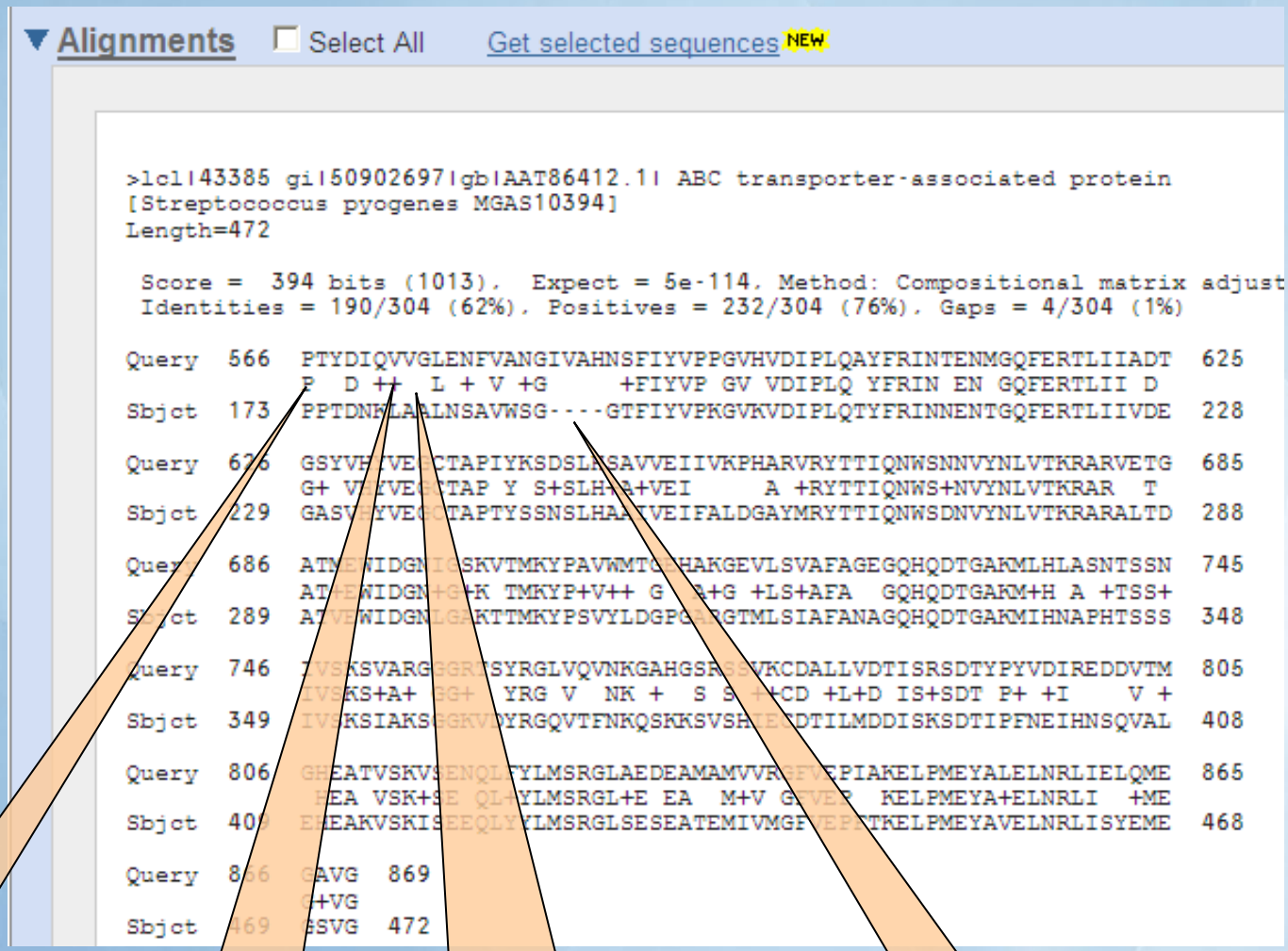
Bl2seq results



Match **Similarity** **Dissimilarity** **Gaps**

MSA input: multiple sequence Fasta file

>gi|4504351|ref|NP_000510.1| delta globin [Homo sapiens]
MVHLTPEEKTAVNALWGKVNVDAVGGEALGRLLVVYPWTQRFFESFGDLSSPDAVMGNPKVKAHGKKVLG
AFSDGLAHLDNLKGTFSQLSELHCDKLHVDPENFRLLGNVLVCVLARNFGKEFTPQMQAAYQKVVAGVAN
ALAHKYH

>gi|4504349|ref|NP_000509.1| beta globin [Homo sapiens]
MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKVKAHGKKVLG
AFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVAN
ALAHKYH

>gi|4885393|ref|NP_005321.1| epsilon globin [Homo sapiens]
MVHFTAEEKAAVTSLWSKMNVEEAGGEALGRLLVVYPWTQRFFDSFGNLSSPSAILGNPKVKAHGKKVLT
SFGDAIKNMDNLKPAFAKLSELHCDKLHVDPENFKLLGNVMVIILATHFGKEFTPEVQAAWQKLVSAVAI
ALAHKYH

>gi|6715607|ref|NP_000175.1| G-gamma globin [Homo sapiens]
MGHFTEEDKATITSLWGKVNVEDAGGETLGRLLVVYPWTQRFFDSFGNLSSASAIMGNPKVKAHGKKVLT
SLGDAIKHLDDLKGTFAQLSELHCDKLHVDPENFKLLGNVLVTVLAIHFGKEFTPEVQASWQKMVTGVAS
ALSSRYH

>gi|28302131|ref|NP_000550.2| A-gamma globin [Homo sapiens]
MGHFTEEDKATITSLWGKVNVEDAGGETLGRLLVVYPWTQRFFDSFGNLSSASAIMGNPKVKAHGKKVLT
SLGDATKHLDDLKGTFAQLSELHCDKLHVDPENFKLLGNVLVTVLAIHFGKEFTPEVQASWQKMVTAVAS
ALSSRYH

>gi|4885397|ref|NP_005323.1| hemoglobin, zeta [Homo sapiens]
MSLTKTERTIIVSMWAKISTQADTIGTETLERLFLSHPQTKTYFPHFDLHPGSAQLRAHGSKVVAAVGDA
VKSIDDIGGALSKLSELHAYILRVDPVNFKLLSHCLLVTLAARFPADFTAEAHAAWDKFLSVVSSVLTEK
YR

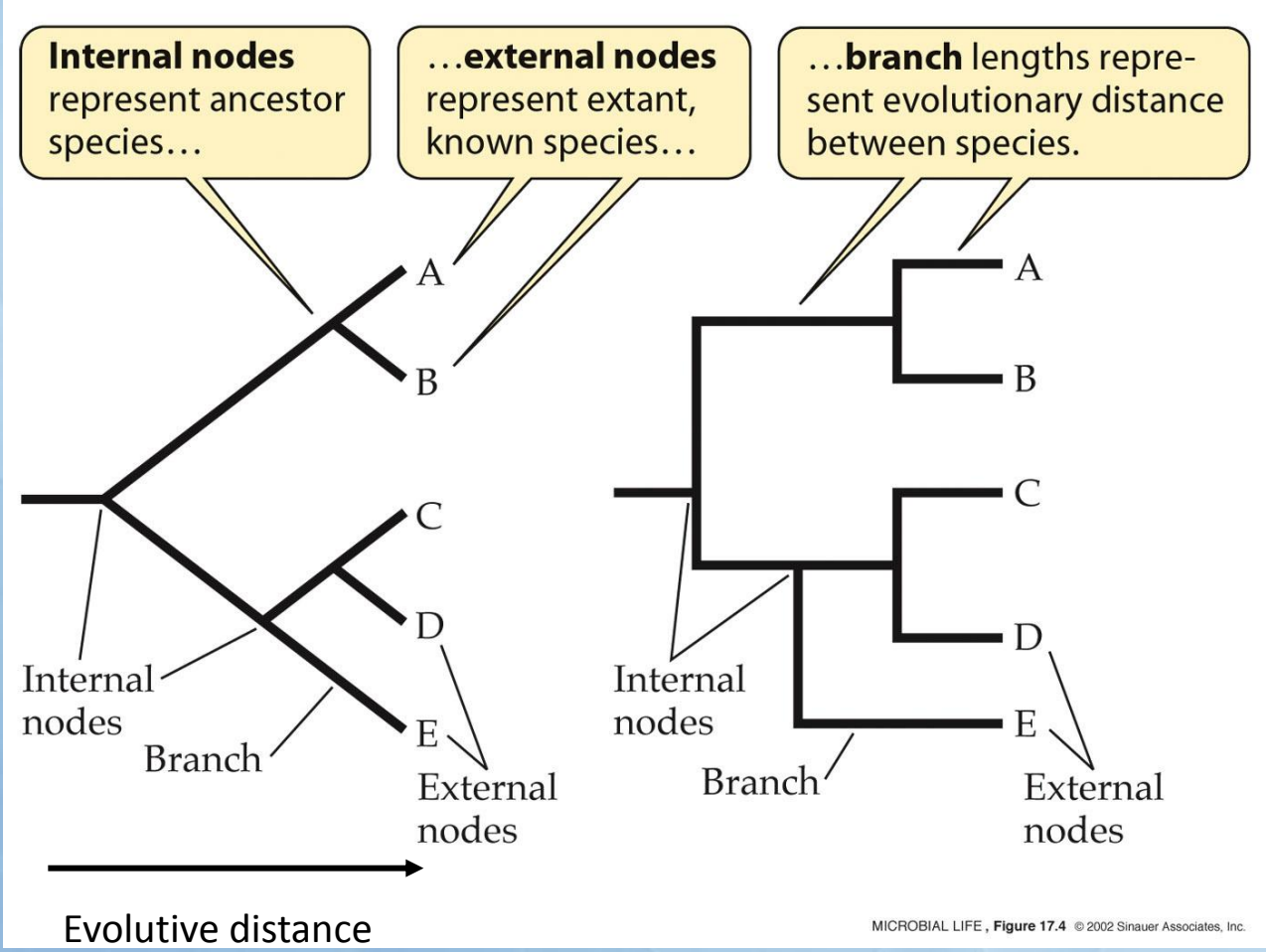
Query type: AA or DNA?

- For coding sequences, AA (protein) data are better
  - Selection operates most strongly at the protein level → the homology is more evident
  - AA – 20 char' alphabet DNA - 4 char' alphabet

↓

lower chance of random homology for AA

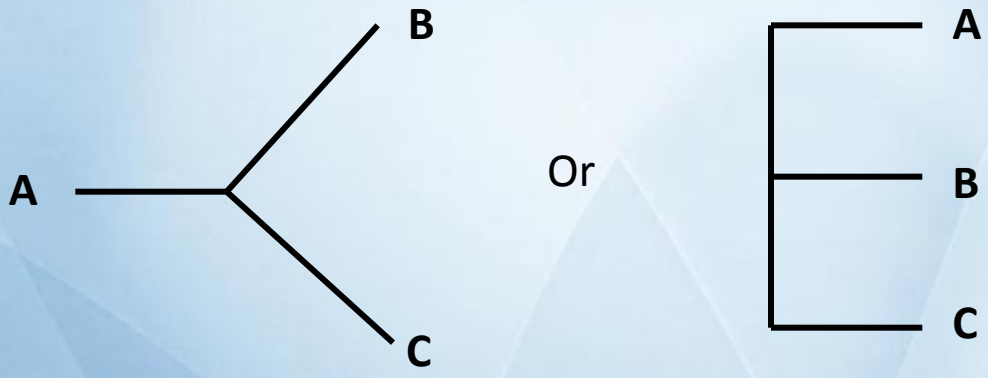
Phylogenetic trees



Phylogenetic trees

Rooted and unrooted trees

- **Unrooted trees:** compare one feature of a group of related organisms



Only one shape for a tree with 3 species

Distance matrix: UPGMA

- **UPGMA = unweighted pair group method with arithmetic mean**

(A) UPGMA method

Table shows sequence of nine-base region of rRNAs of four strains.

Organism (strain)	Site number 1	2	3	4	5	6	7	8	9
a	G	C	G	G	A	C	A	A	A
b	G	A	C	G	C	C	A	A	G
c	G	A	A	A	U	C	U	A	A
d	G	A	A	A	G	C	U	A	G

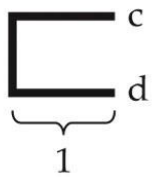
1 Construct matrix showing relatedness between strains.

First matrix

	a	b	c
b	$d_{ab} = 4$	—	—
c	$d_{ac} = 5$	$d_{bc} = 5$	—
d	$d_{ad} = 6$	$d_{bd} = 4$	$d_{cd} = 2$

2 Diagram relatedness between strains.

Beginning tree



where $\frac{d_{cd}}{2} = 1$

MICROBIAL LIFE, **Figure 17.6 (Part 1)** © 2002 Sinauer Associates, Inc.

Distance matrix: UPGMA

c & **d** are considered as one unit

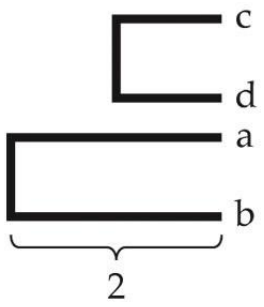
Second matrix

3 Construct matrix to assess distance between (cd) and (a and b).

	(cd)	a
a	$d_{(cd)a} = {}^{11}/_{2}$	—
b	$d_{(cd)b} = {}^{9}/_{2}$	$d_{ab} = 4$

Second tree

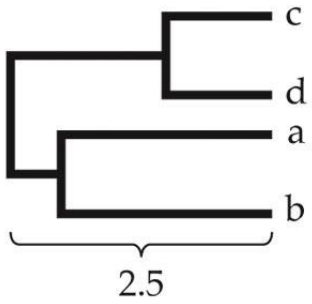
4 Determine and diagram their relatedness.



where $\frac{d_{ab}}{2} = 2$

Final tree

5 Determine relatedness between cd and a and b.

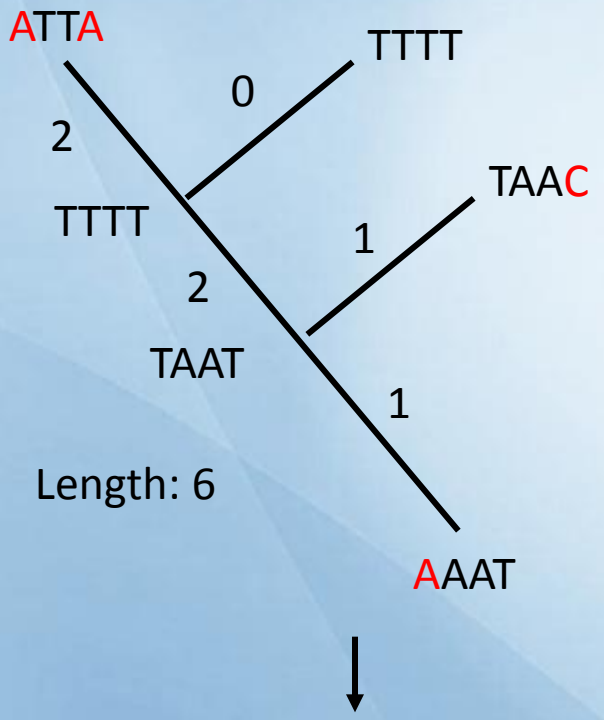


where $d_{(cd)(ab)} = \frac{({}^{11}/_{2} + {}^{9}/_{2})/2}{2} = 2.5$

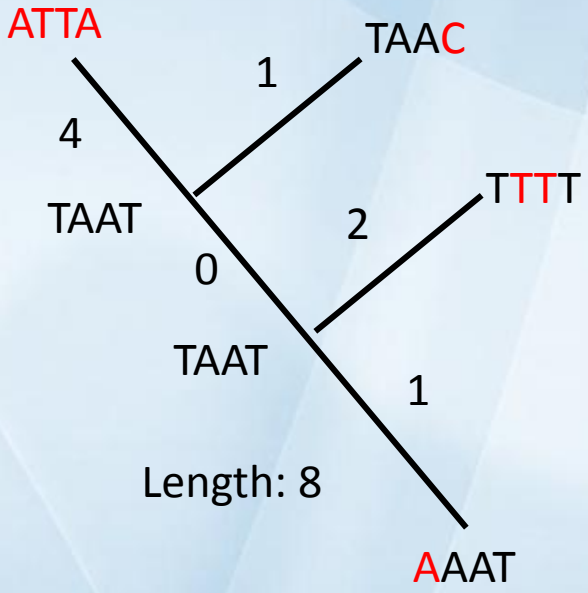
MICROBIAL LIFE, **Figure 17.6 (Part 2)** © 2002 Sinauer Associates, Inc.

Other method: maximum parsimony

Sequence 1: ATTA
Sequence 2: TAAC
Sequence 3: AAAT
Sequence 4: TTTT



Length: 6

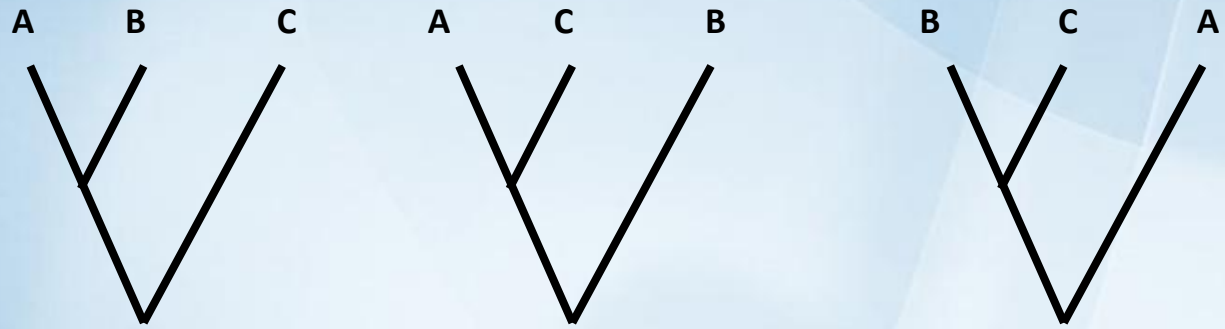


Length: 8

Most likely tree

The alignment problem

- What happens when a sequence alignment is wrong?



A: AGT
B: AT
C: ATC

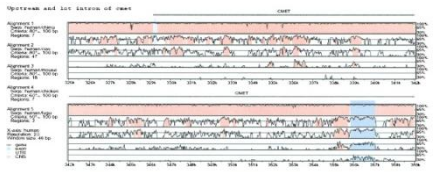
A: AGT
B: A -T
C: ATC

A: AGT -
B: A -T -
C: A -TC

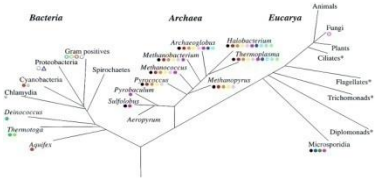
A: AGT
B: AT -
C: ATC

Central role of multiple alignments

Comparative genomics

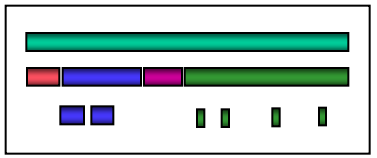


Phylogenetic studies

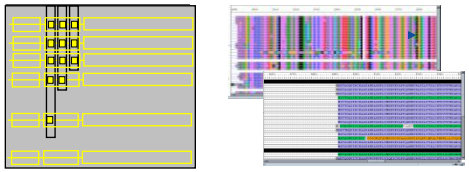


Hierarchical function annotation:

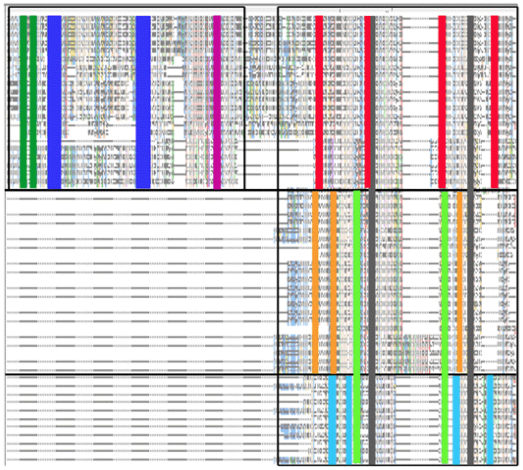
homologs, domains, motifs



Gene identification, validation

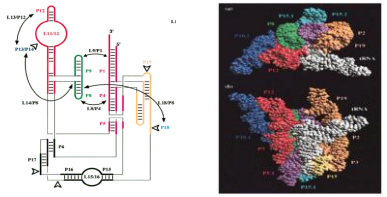


Multiple alignment

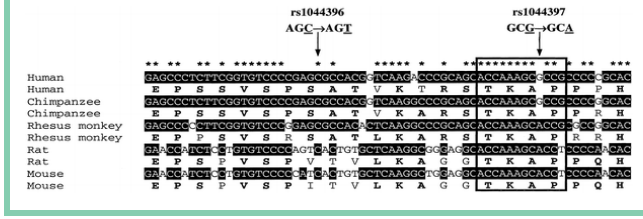


Structure comparison, modelling

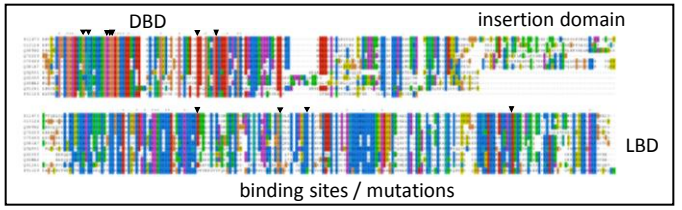
RNA sequence, structure, function



Human genetics, SNPs



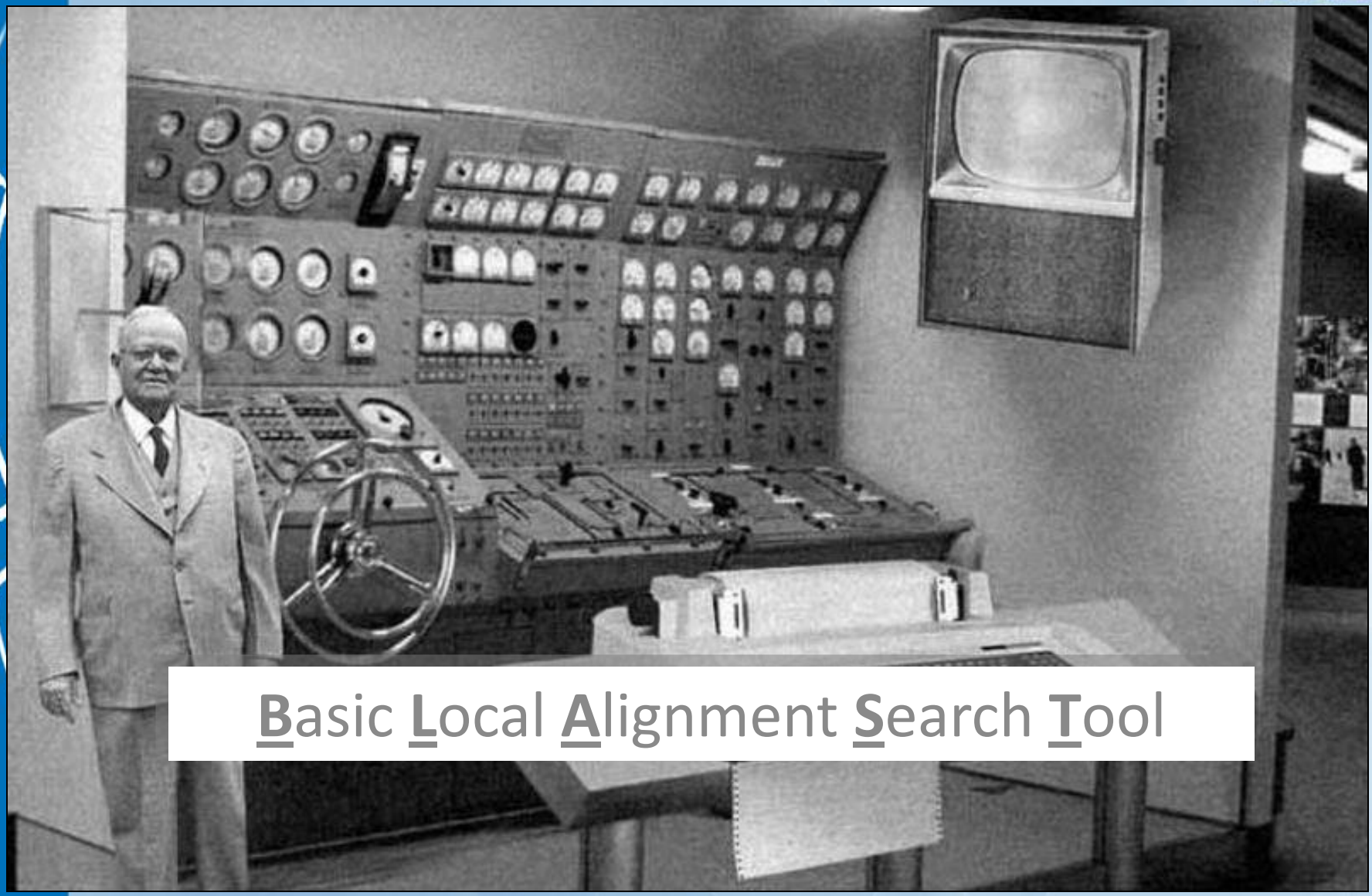
Therapeutics, drug design



Most important sequence databases

- Genbank– maintained by USA National Center for Biology Information (NCBI)
  - All biological sequences
    - www.ncbi.nlm.nih.gov/Genbank/GenbankOverview.html
  - Genomes
    - www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?db=Genome
- Swiss-Prot - maintained by EMBL- European Bioinformatics Institute (EBI)
  - Protein sequences
    - www.ebi.ac.uk/swissprot/

Sequence Similarity Searching



Basic **L**ocal **A**lignment **S**earch **T**ool