Basic Bioinformatics, Sequence Alignment, and Homology

Biochemistry Boot Camp 2019
Session #10
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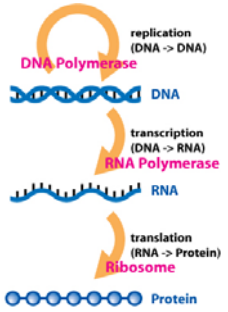
* BLAST slides have been adapted from an earlier presentation by W. Shane Sanders.

Biology Review

- Genome is the genetic material of an organism, normally DNA but RNA possible (viruses)
- Central Dogma:
  - DNA → RNA → Protein



The Central Dogma of Molecular Biology

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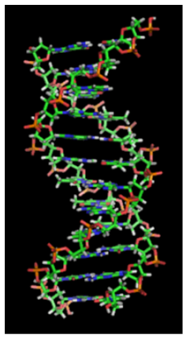
Primary Structure (Sequence)

- **DNA and Proteins are chemically complex**, but their "alphabets" are rather simple.
  - 4 nucleobases (A, C, T, G)
  - 20 amino acids
- DNA sequences are represented from 5' to 3'



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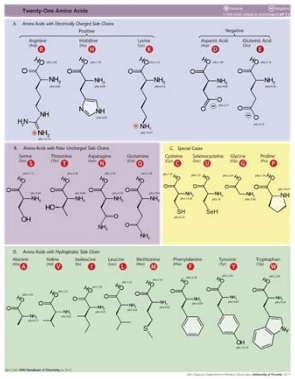
Primary Structure (Sequence)

- **DNA and Proteins are chemically complex**, but their "alphabets" are rather simple.
  - 4 nucleobases (A, C, T, G)
  - 20 amino acids
- Protein sequences are represented from NT to CT



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Storing Sequences

- GenBank (*.gb| *.genbank)
  - National Center for Biotechnology's (NCBI) Flat File Format (text)
  - Provides a large amount of information about a given sequence record
  - http://www.ncbi.nlm.nih.gov/Sitemap/samplerecord.html
  - We've seen this before! (Remember NCBI Protein result?)
- **FASTA (*.fasta | *.fa)**
  - Pronounced "FAST-A"
  - Simple text file format for storing nucleotide or peptide sequences
  - Each record begins with a single line description starting with ">" and is followed by one or more lines of sequence
- FASTQ (*.fastq | *.fq)
  - Pronounced "FAST-Q"
  - Text based file format for storing nucleotide sequences and their corresponding quality scores
  - Quality scores are generated as the nucleotide is sequenced and correspond to a probability that a given nucleotide has been correctly sequenced by the sequencer
- **Text files are also okay in many cases.**

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Storing Sequences

- FASTA format
- Can represent nucleotide sequences or peptide sequences using single letter codes

```
>gi|5524211|gb|AAD44166.1| cytochrome b [Elephas maximus maximus]
LCLYTHIGRNIYYGSYLYSETWNTGIMLLLITMATAFMGYVLPWGQMSFWGATVITNLFSAIPYIGTNLV
EWIWGGFSVDKATLNRFFAFHFILPFTMVALAGVHLTFLHETGSNNPLGLTSDSDKIPFHPYYTIKDFLG
LLILILLLLLLALLSPDMLGDPDNHMPADPLNTPLHIKPEWYFLFAYAILRSVPNKLGGVLALFLSIVIL
GLMPFLHTSKHRSMMLRPLSQALFWTLTMDLLTLTWIGSQPVEYPYTIIGQMASILYFSIILAFLPIAGX
IENY
```

- FASTQ format
- Represents nucleotide sequences and their corresponding quality scores

```
@SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65
```

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Sequence Alignment

Sequence alignment is the procedure of comparing two (pairwise) or more (multiple) sequences and searching for a series of individual characters or character patterns that are the same in the set of sequences.

- **Global alignment** – find matches along the entire sequence (use for sequences that are quite similar)
- **Local alignment** – finds regions or islands of strong similarity (use for comparing less similar regions [finding conserved regions])

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Sequence Alignment

Sequence 1: GARVEY

Sequence 2: AVERY

Global Alignment:

GARVE-Y

-A-VERY

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Global Sequence Alignment

- EMBOSS Needle
  http://www.ebi.ac.uk/Tools/psa/emboss_needle/
  – Command line version also available
- Alternative: Biopython (library for the python programming language)
- **Example:** Human vs. Nematode Calmodulin (global sequence #1 and #2)

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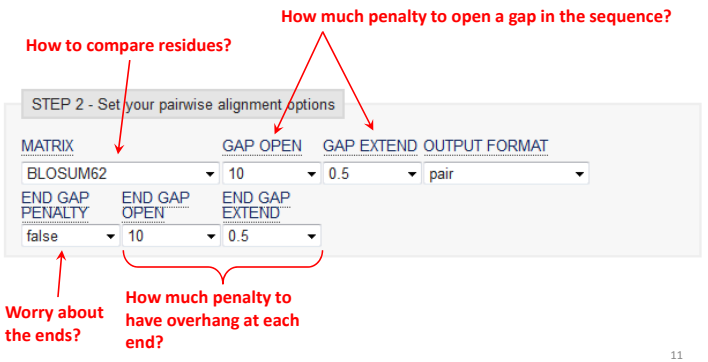
Global Sequence Alignment

- EMBOSS Needle Options:



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Global Sequence Alignment

```
# Length: 149
# Identity:     146/149 (98.0%)
# Similarity:   147/149 (98.7%)
# Gaps:           0/149 ( 0.0%)
# Score: 745.0

Human               1 MADQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMRSLGQNPTEAELQ     50
                      ||||||||||||||||||||||||||||||||||||||||||||||||||
Nematode            1 MADQLTEEQIAEFKEAFSLFDKDGDGTITTKELGTVMRSLGQNPTEAELQ     50

Human              51 DMINEVDADGNGTIDFPEFLTMMARKMKDTDSEEEIREAFRVFDKDGNGY    100
                      ||||||||||||||||||||||||||||||||||||||||||||||||||:
Nematode           51 DMINEVDADGNGTIDFPEFLTMMARKMKDTDSEEEIREAFRVFDKDGNGF    100

Human             101 ISAAELRHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEFVQMMTAK    149
                      ||||||||||||||||||||||||||||||||||||||||||.|||.|
Nematode          101 ISAAELRHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEFVTMMTTK    149
```

Percent Identity and Similarity quantify alignment.

- Pretty darn similar!

Identical residues shown with |, similar residues with : and ., and blanks represent dissimilar residues.

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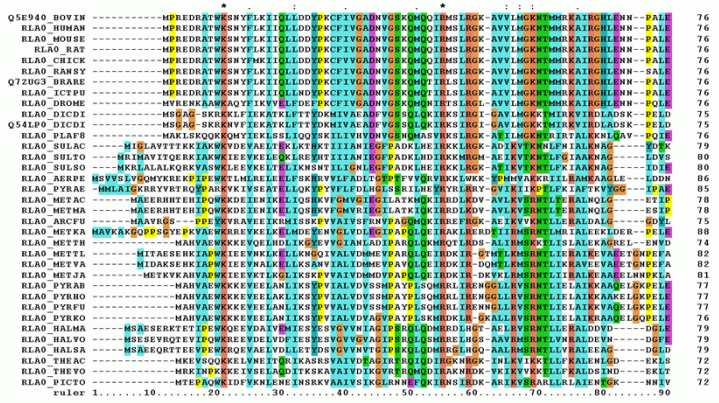
Multiple Sequence Alignment

- Align many sequences simultaneously, normally from multiple organisms
- Mathematically much more challenging, and requires assumptions about data analysis
- Results can be used to generate phylogenetic tree
  – https://www.ebi.ac.uk/Tools/msa/clustalo/
- Example software: MEGA, ClustalX
  http://www.megasoftware.net/
  http://www.clustal.org/

MEGA Molecular Evolutionary Genetics Analysis

MSA Example



MSA of Ribosomal Protein P0 from Wikipedia, "Multiple Sequence Alignment"

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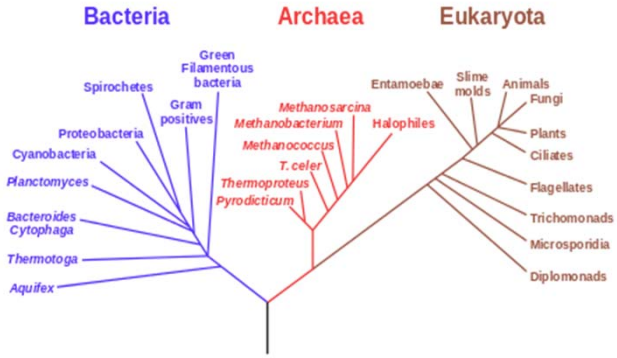
MSA-Derived Phylogenetic Tree



Phylogenetic Tree derived from ribosomal proteins, Wikipedia "Phylogenetic Tree"

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Why Sequence Alignment?

1. To determine possible functional similarity.
2. For 2 sequences:
   a. If they're the same length, are they almost the same sequence? (global alignment)
3. For 2 sequences:
   a. Is the prefix of one string the suffix of another? (contig assembly)
4. Given a sequence, has anyone else found a similar sequence?
5. To identify the evolutionary history of a gene or protein.
6. To identify genes or proteins.

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BLAST:
Basic Local Alignment Search Tool

- A tool for determining sequence similarity
- Originated at the National Center for Biotechnology Information (NCBI)
- Sequence similarity is a powerful tool for identifying unknown sequences
- BLAST is fast and reliable
- BLAST is flexible

http://blast.ncbi.nlm.nih.gov/

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Flavors of BLAST

- **blastn** – searches a nucleotide database using a nucleotide query
  DNA/RNA sequence searched against DNA/RNA database
- **blastp** – searches a protein database using a protein query
  Protein sequence searched against a Protein database
- **blastx** – search a protein database using a translated nucleotide query
  DNA/RNA sequence -> Protein sequence searched against a Protein database
- **tblastn** – search a translated nucleotide database using a protein query
  Protein sequence searched against a DNA/RNA sequence database -> Protein sequence database
- **tblastx** – search a translated nucleotide database using a translated nucleotide query
  DNA/RNA sequence -> Protein sequence searched against a DNA/RNA sequence database -> Protein sequence database

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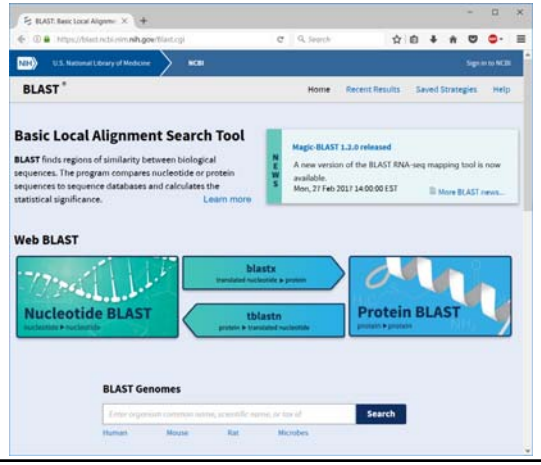
BLAST Main Page



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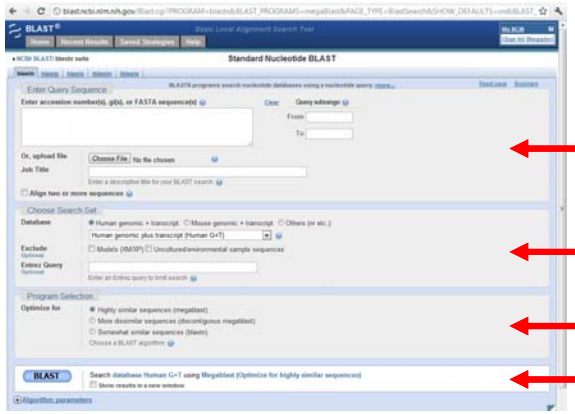
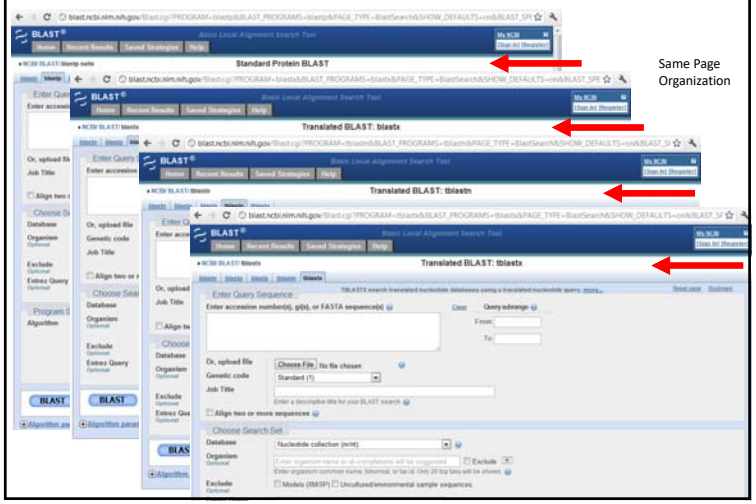


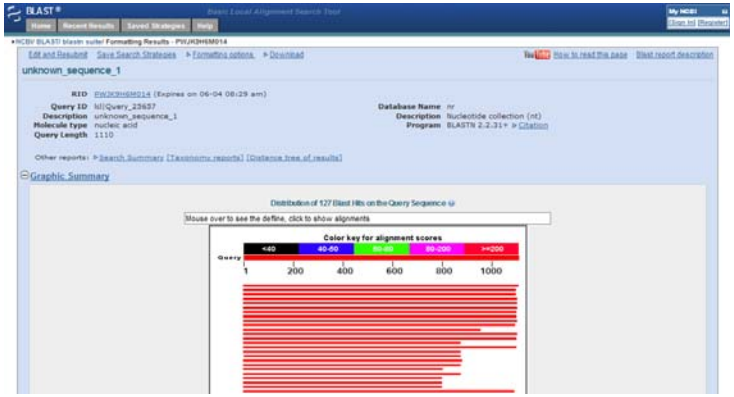


BLAST Example

- What gene is this?

```
>unknown_sequence_1
TGATGTCAAGACCCTCTATGAGACTGAAGTCTTTTCTACCGACTTCTCCAACATTTCTGCAGCCAAGCAG
GAGATTAACAGTCATGTGGAGATGCAAACCAAAGGGAAAGTTGTGGGTCTAATTCAAGACCTCAAGCCAA
ACACCATCATGGTCTTAGTGAACTATATTCACTTTAAAGCCCAGTGGGCAAATCCTTTTGATCCATCCAA
GACAGAAGACAGTTCCAGCTTCTTAATAGACAAGACCACCACTGTTCAAGTGCCCATGATGCACCAGATG
GAACAATACTATCACCTAGTGGATATGGAATTGAACTGCACAGTTCTGCAAATGGACTACAGCAAGAATG
CTCTGGCACTCTTTGTTCTTCCCAAGGAGGGACAGATGGAGTCAGTGGAAGCTGCCATGTCATCTAAAAC
ACTGAAGAAGTGGAACCGCTTACTACAGAAGGGATGGGTTGACTTGTTTGTTCCAAAGTTTTCCATTTCT
GCCACATATGACCTTGGAGCCACACTTTTGAAGATGGGCATTCAGCATGCCTATTCTGAAAATGCTGATT
TTTCTGGACTCACAGAGGACAATGGTCTGAAACTTTCCAATGCTGCCCATAAGGCTGTGCTGCACATTGG
TGAAAAGGGAACTGAAGCTGCAGCTGTCCCTGAAGTTGAACTTTCGGATCAGCCTGAAAACACTTTCCTA
CACCCTATTATCCAAATTGATAGATCTTTCATGTTGTTGATTTTGGAGAGAAGCACAAGGAGTATTCTCT
TTCTAGGGAAAGTTGTGAACCCAACGGAAGCGTAGTTGGGAAAAAGGCCATTGGCTAATTGCACGTGTGT
ATTGCAATGGGAAATAAATAAATAATATAGCCTGGTGTGATTGATGTGAGCTTGGACTTGCATTCCCTTA
TGATGGGATGAAGATTGAACCCTGGCTGAACTTTGTTGGCTGTGGAAGAGGCCAATCCTATGGCAGAGCA
TTCAGAATGTCAATGAGTAATTCATTATTATCCAAAGCATAGGAAGGCTCTATGTTTGTATATTTCTCTT
TGTCAGAATACCCCTCAACTCATTTGCTCTAATAAATTTGACTGGGTTGAAAAATTAAAA
```


BLAST Results



Interpreting BLAST Results

- **Max Score** – how well the sequences match
- **Total Score** – includes scores from non-contiguous portions of the subject sequence that match the query
- **Bit Score** – A log-scaled version of a score
  - Ex. If the bit-score is 30, you would have to score on average, about 2^{30} = 1 billion independent segment pairs to find a score matching this score by chance. Each additional bit doubles the size of the search space.
- **Query Coverage** – fraction of the query sequence that matches a subject sequence
- **E value** – how likely an alignment can arise by chance
- **Max ident** – the match to a subject sequence with the highest percentage of identical bases

Installing BLAST Locally

Executables and documentation available at:
ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/

Documentation:
http://www.ncbi.nlm.nih.gov/books/NBK1762/

Aligning via Structure

- So far we've focused on *sequence* alignment: looking at the primary (DNA or protein) sequence
- What about *structural* alignment? (Think shape or similar domains)
- VAST (Vector Alignment Search Tool) at NCBI: https://structure.ncbi.nlm.nih.gov/Structure/VAST/vast.shtml

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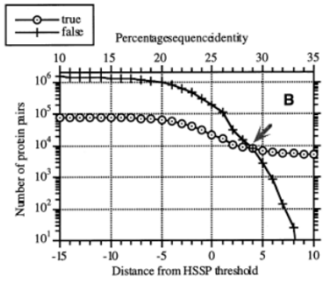
Homology Modeling

- Proteins with similar sequences tend to have similar structures.
- When sequence identify is greater than ~25%, this rule is almost guaranteed
  - Exception: See Lauren Perskie-Porter, Phil Bryan and "fold switching"
- Can we predict structures?



Below ~28% sequence identity, the number of structurally dissimilar aligned pairs explodes.

Rost, *Prot. Eng.* 12(2): 85-94

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What is Homology Modeling?

- **Consider:** Protein with known sequence, but unknown structure
- Use sequence alignment (protein BLAST) to identify similar sequences with known structures
  - These are termed "template structures"
- "Map" unknown sequence onto known backbone
  - Side chains may be more ill-defined: it's a model!

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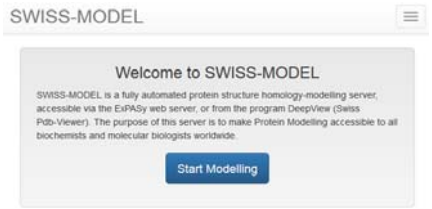
Homology Modeling Servers: **SWISS-MODEL**



- Web page: http://swissmodel.expasy.org/
- Fastest option, can take less than 5 minutes
- Final model typically based on a single template (users can upload their own)

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Homology Modeling Servers: **Phyre²**

Phyre²

Protein Homology/analogY Recognition Engine V 2.0

- Web page: http://www.sbg.bio.ic.ac.uk/phyre2/
- Trade off: can take 1-2 hours depending on server demand, but better structures
- Uses multiple templates, users can exclude files

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Homology Modeling Servers: **I-TASSER**

I-TASSER
Protein Structure & Function Predictions
(The server completed predictions for 472764 proteins submitted by 112444 users from 144 countries)
(The template library was updated on 2019/06/02)

- Web page: http://zhanglab.ccmb.med.umich.edu/I-TASSER/
- Slowest option by far; can take a day or more
- Uses multiple templates and performs sophisticated refinement

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Homology Modeling Example

- Sequence for Pin1 protein:

```
MADEEKLPPG WEKRMSRSSG RVYYFNHITN ASQWERPSGN SSSGGKNGQG
EPARVRCSHL LVKHSQSRRP SSWRQEKITR TKEEALELIN GYIQKIKSGE
EDFESLASQF SDCSSAKARG DLGAFSRGQM QKPFEDASFA LRTGEMSGPV
FTDSGIHIIL RTE
```

- Use BLAST to identify a homologous cis-trans prolyl isomerase in *Methanocorpusculum labreanum*

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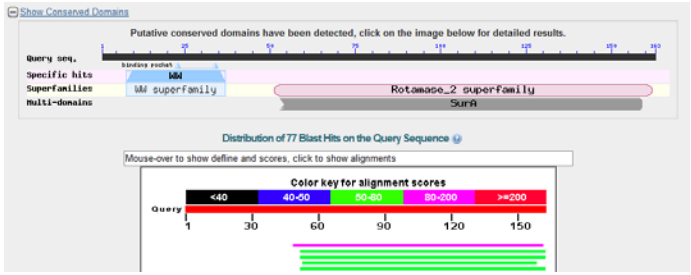
Homology Modeling Example

- Initial BLASTp result:



- Sequence (only second domain found):

```
MVRVKASHIL VKTEAQAKEI MQKISAGDDF AKLAKMYSQC PSGNAGGDLG
YFGKGQMVKP FEDACFKAKA GDVVGPVKTQ FGWHIIKVTD IKN
```

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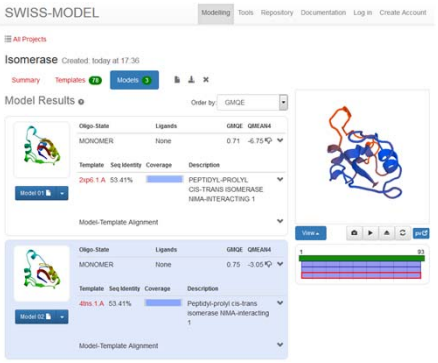
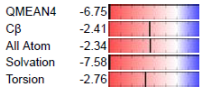
Result: SWISS-MODEL

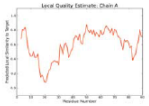


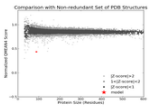
- We'll do this model in class

Result: SWISS-MODEL

Model #01	File	Built with	Oligo-State	Ligands	GMQE	QMEAN4
	PDB	ProMod Version 3.70.	MONOMER	None	0.71	-6.75



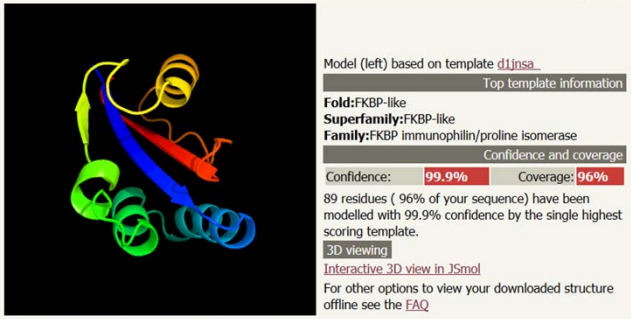




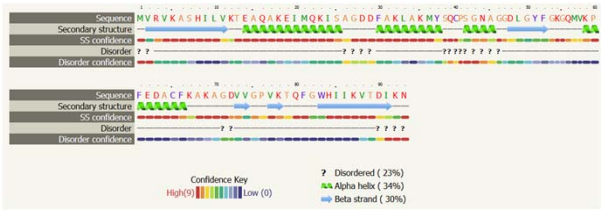
Template	Seq Identity	Oligo-state	Found by	Method	Resolution	Seq Similarity	Range	Coverage	Description
2xp6.1.A	53.41	monomer	BLAST	X-ray	1.90Å	0.45	3 - 90	0.95	PEPTIDYL-PROLYL CIS-TRANS ISOMERASE NIMA-INTERACTING 1

Ligand	Added to Model	Description
12P	X - Binding site not conserved.	DODECAETHYLENE GLYCOL
4G2	X - Binding site not conserved.	2-(3-CHLORO-PHENYL)-5-METHYL-1H-IMIDAZOLE-4-CARBOXYLIC ACID

Result: Phyre^2

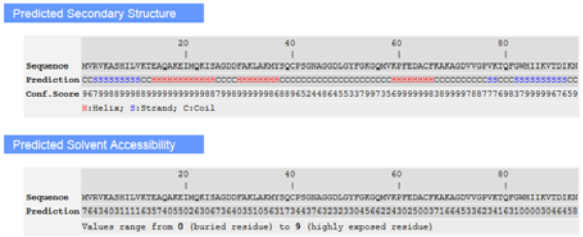


Result: Phyre^2



- Download entire result, which is a duplicate of the website, can be viewed here:
  http://folding.chemistry.msstate.edu/files/bootcamp/phyre2/summary.html
- Final result is called `final.casp.pdb`

Result: I-TASSER



- Results available at:
  http://folding.chemistry.msstate.edu/files/bootcamp/itasser/
- Final result is called `model1.pdb`

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Comparison of Results

- **Download the following PDBs from the Boot Camp Website:**
  - 1pin.pdb – Original Pin1 Structure
  - swiss.pdb – SWISS-MODEL Result
  - phyre2.pdb – Phyre² Result
  - itasser.pdb – I-TASSSER Result
- PyMOL can help us here using the "align" command

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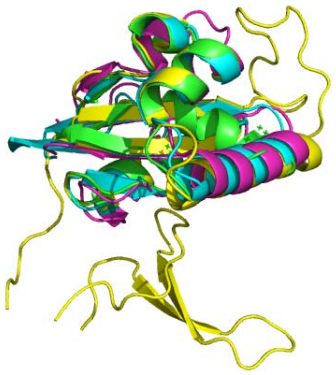
Comparison of Results



- Colors:
  - Original Pin1
  - SWISS-MODEL
  - Phyre²
  - I-TASSER
- **Important:** How much side chain accuracy do I need?

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Other Resources:

- EMBL-EBI (European Bioinformatics Institute) - http://www.ebi.ac.uk/
- DDBJ (DNA Data Bank of Japan) - http://www.ddbj.nig.ac.jp/
- NCBI's Sequence Read Archive (SRA) - http://www.ncbi.nlm.nih.gov/sra
- UCSC Genome Browser: http://genome.ucsc.edu/
- IGBB's Useful Links Page - http://www.igbb.msstate.edu/links.php

Many, many more available online, just search.

Summary

- Sequence alignment is an important tool for searching and understanding how proteins are related
- BLAST can be used to search for similar sequences in large protein/DNA databases (and also works in tools like the PDB)
- Homology modeling can be helpful way to understand structures of unknown proteins

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