



The Institute of Endemic Diseases
University of Khartoum

Bioinformatics

Basic Bioinformatics

28 December 2017 - 04 January 2018

Basic Bioinformatics Sessions

Practical 5: Secondary Structure Prediction

Saturday 23 December 2017

Protein Structure

In this exercise, the plan is to look briefly at one of the most complete ways to predict the **Secondary Structure of a Protein** (or **Family of Proteins**) and to then glance at how a given **Protein Tertiary Structure** could be retrieved from the **3D Structure Databases** and examined.

Predicting Protein Secondary Structure.

Feature key	Position(s)	Description	Actions	Graphical view	Length
Beta strand ⁱ	6 – 8	Combined sources			3
Beta strand ⁱ	14 – 16	Combined sources			3
Helix ⁱ	23 – 34	Combined sources			12
Helix ⁱ	39 – 46	Combined sources			8
Helix ⁱ	50 – 63	Combined sources			14
Beta strand ⁱ	77 – 79	Combined sources			3
Helix ⁱ	81 – 93	Combined sources			13
Helix ⁱ	99 – 108	Combined sources			10
Turn ⁱ	114 – 116	Combined sources			3
Helix ⁱ	120 – 133	Combined sources			14
Helix ⁱ	219 – 229	Combined sources			11
Helix ⁱ	237 – 246	Combined sources			10
Helix ⁱ	251 – 275	Combined sources			25

As ever, we use the **PAX6** protein as an example. Evidence from various sources suggests that the **PAX6** protein has **9** helices arranged in triplets, plus a few beta strands.

As a reminder, I show here the relevant section from the **UniprotKB Feature Table**. The helical triplets are involved in binding. **2** triplets are to be found in the paired box region, the other in the homeobox a little further along.

Here we will use one of the most sophisticated methods available, to predict the secondary structure we already know, from from primary sequence. Out of curiosity, I will compare the prediction with that of one of the earlier prediction methods (still used, but although faster, significantly less accurate than modern methods).

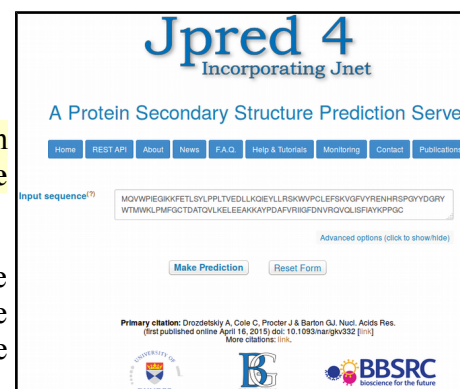
The service considered by many to offer the most effective method of predicting secondary structure is called **Jpred**. This is developed by the **Barton Group** now located at Dundee University. Over **80%** accuracy is claimed for **Jpred** predictions. Due to the inherent imprecision in defining the end positions of secondary structure elements, **80%** is pretty much as good as is practically possible.

Go to the **Barton Group** web site at:

<http://www.compbio.dundee.ac.uk>

and follow the link to the **Jpred 4** server. Copy and paste the **PAX6** protein (from the file **pax6_human.fasta**) into the appropriate text box. Click on **Make Prediction**.

With alacrity, **JPred** will report several hits with proteins of known **3D** structure (using **blast** against a database of proteins of known **3D** structure). Links are offered to a number of entries in the **PDB** structure database. At least **2** of the **PDB** entries listed should be familiar.



Match found in PDB

The sequence you submitted is similar to those with known structure. These may provide a more accurate secondary structure assignment than a JPred prediction.

If you still want to carry out a Jpred prediction click **continue**

Hits found

Show **25** entries

PDB	Chain	Description	Blast E-value
6pax	A	HOMEBOX PROTEIN PAX-6	1e-69
1mdm	A	PAIRED BOX PROTEIN PAX-5	9e-53
1k78	I	Paired Box Protein Pax5	9e-53
1k78	E	Paired Box Protein Pax5	9e-53
1k78	A	Paired Box Protein Pax5	9e-53
2k27	A	Paired box protein Pax-8	4e-52
1pdn	C	PROTEIN (PRD PAIRED)	2e-41
2cue	A	Paired box protein Pax6	2e-32

least, this has to be a fine idea. A **Multiple Sequence Alignment (MSA)** of related proteins will typically represent far more evidence for prediction than any single protein.

Jpred proposes that it really does not make sense to continue. After all, if the **3D** structure is effectively known, why predict (guess?) the **2D** structure? The response to this challenge being a petulant “**Because we want to!**”

Click purposefully on the **Continue** button. **JPred**, with a small sigh of exasperation, will submit your job and tells you how busy it is. **Jpred** typically takes a while as it has much to consider.

Jpred will use **PSI-Blast** to align your sequence with all sequences deemed to be homologous, from a particularly appropriate database. **Jpred** then makes its structure predictions based on an aligned “family” of proteins, rather than just one individual sequence. Intuitively at

JPred presents the results of running two secondary structure predictions, using the program **JNET**, based on two different representations of the alignment (**HMM** and **PSSM**, similar ideas [that will be discussed at some point](#)). Predicted helices are represented as red blocks, predicted beta sheets as green arrows. A consensus prediction is presented (**jnetpred**) as an indication of prediction confidence (**JNETCONF**). Algorithms are also run to predict **coiled coils** (**Lupas**, with window sizes **21**, **14**, **28**). The first view of the results offered is a graphical overview aligned with your original single sequence.

The full key to all the abbreviations used (and more information about **JNet**) can be displayed by [clicking on the details on acronyms used](#) link.

For a fuller view, elect to **View results in Jalview**¹. You will arrive at a page inviting you to select from various viewing options. The options are explained clearly, but to save you time reading and pain deciding, I suggest you [go for Option 1](#) for the clearest view. This option does not confuse the picture by gapping your query sequence (and thus making it more difficult to associate structure predictions with regions of the **PAX6** protein) and does not force you to look at the entire, huge, **MSA** generated by **PSI-Blast**.

Jalview presents something very similar to the original view of the **Jpred** results. This time though, the most significant part of the **PSI-Blast** **MSA** from which the predictions were computed is displayed, if rather blandly.

To highlight the conserved regions of the alignment, some colour is required. **Jalview**, offers a number of colouring strategies. I refer you to the **Help** for the full story. Here I will choose what I think is a revealing option with minimal explanation².

From the **Jalview Colour** pull down menu, select **BLOSUM62 Score**, to suggest that the inclination to colour any amino acid of the **MSA** be determined from its **BLOSUM 62 Score** with the corresponding **Consensus** sequence residue and the degree of conservation at that alignment position. A considerable number of conserved **MSA** positions around the homeobox region will now be coloured various shades of blue.

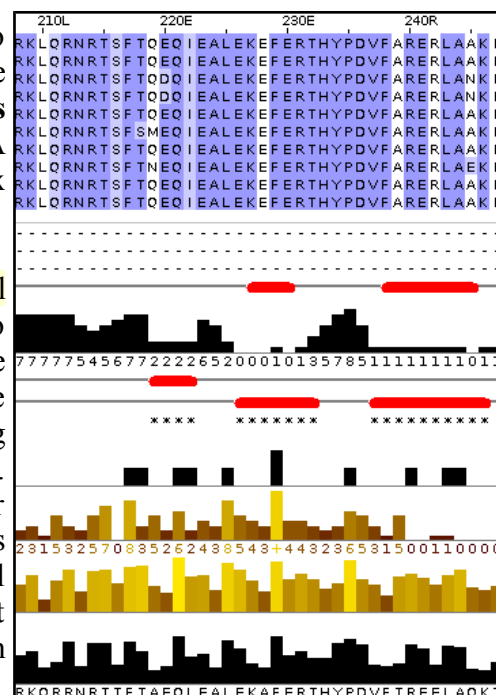
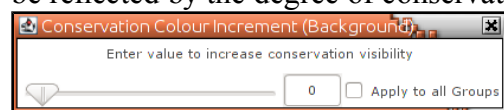
In order to vary the subtlety of your display, from the **Jalview Colour** pull down menu, select **By Conservation**, thus electing for the colour intensity to be reflected by the degree of conservation for each **MSA** column. A jolly little slider bar will leap forward. At the default setting (**30**), the colouring becomes somewhat more subtle.

Slide the bar to and fro to achieve the delusion that you have control over things. Terminate your oscillations with the minimum value selected, thus demanding that any slight odour of conservation should elicit a maximal colour burst! Appropriate as the interestingly conserved regions are thus most clearly distinguishable. Ignore the reference to **Groups** as none have been specified, so the entire **MSA** is regarded as a single **Group**.

Now, all the regions regarded as vaguely conserved glow enthusiastically blue. Slide along the entire width of the **MSA** and you should clearly see the **Paired Box domain**, **Homeobox domain** and the **compositionally biased C-terminus** are, for the most part, very evident.

The annotation bars below the alignment are as follows:

- Lupas_21, Lupas_14, Lupas_28
Coiled-coil predictions for the sequence. These are binary predictions for each location.
- jnet Burial
Prediction of Solvent Accessibility. levels are
 - 0 - Exposed
 - 3 - 25% or more S.A. accessible
 - 6 - 5% or more S.A. accessible
 - 9 - Buried (<5% exposed)
- JNetPRED
The consensus prediction - helices are marked as red tubes, and sheets as dark green arrows.
- JNetCONF
The confidence estimate for the prediction. High values mean high confidence. prediction - helices are marked as red tubes, and sheets as dark green arrows.
- JNetALIGN
Alignment based prediction - helices are marked as red tubes, and sheets as dark green arrows.
- JNetHMM
HMM profile based prediction - helices are marked as red tubes, and sheets as dark green arrows.
- JNetPSSM
PSSM based prediction - helices are marked as red tubes, and sheets as dark green arrows.
- JNETJURY
A * in this annotation indicates that the JNETJURY was invoked to rationalise significantly different primary predictions.



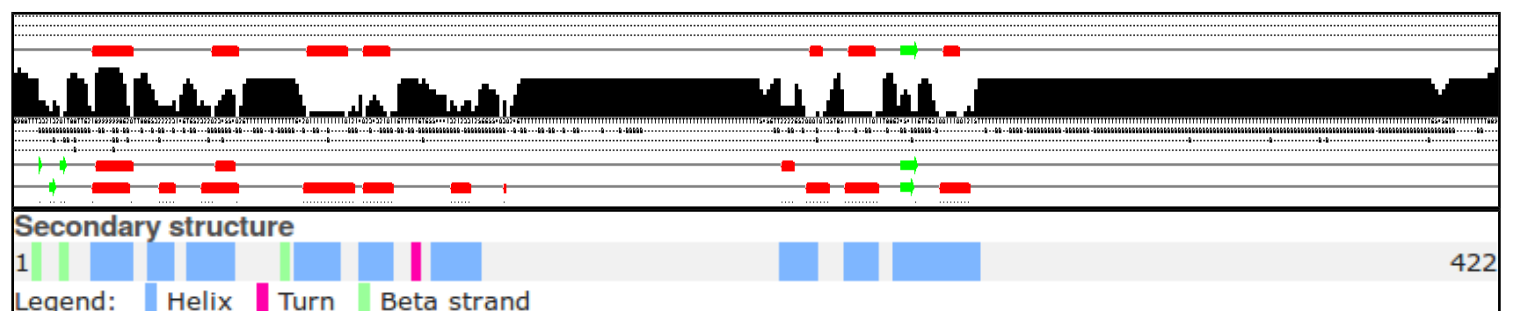
¹ Should that not work, try **Full HTML**.

² I have made some notes on my choice, but they should not really detain you at his point. If you insist, they are [here](#).

[illegible][illegible]

What protein database has **Jpred** chosen to search for protein sequences for the alignment upon which its predictions will be based?

Also, I have lined up the entire prediction with the **Uniprot** Feature Table graphic.



How would you rate the **Jpred** prediction overall?

Protein Tertiary Structure

Protein Data Bank (PDB)

The **Protein Data Bank (PDB)** archive is the major repository of information about the 3D structures of biological molecules, including proteins and nucleic acids. Structures in the archive range from tiny proteins and bits of DNA to complex molecular machines like the ribosome.



In 1998, the **Research Collaboratory for Structural Bioinformatics (RCSB)** became responsible for the management of the **PDB**.

In 2003, the **wwPDB** formed to maintain a single **PDB** archive of macromolecular structural data that is freely and publicly available to the global community. It consists of organizations that act as deposition, data processing and distribution centres for **PDB** data.



PDBe is the European resource for the collection, organisation and dissemination of data on biological macromolecular structures. In collaboration with the other **WorldWide Protein Data Bank (wwPDB)** and **EMDataBank** partners, they work to collate, maintain and provide access to the global repositories of macromolecular structure data (the Protein Data Bank (**PDB**) and Electron Microscopy Data Bank (**EMDB**)).




In the course of the exercises undertaken to this point, you will have already had a look at the **3D** structures for the 2 major domains of the human **PAX6** protein. You might have taken a more direct route to these structures by asking for them directly from the **RCSB PDB** site as follows.

Go to:

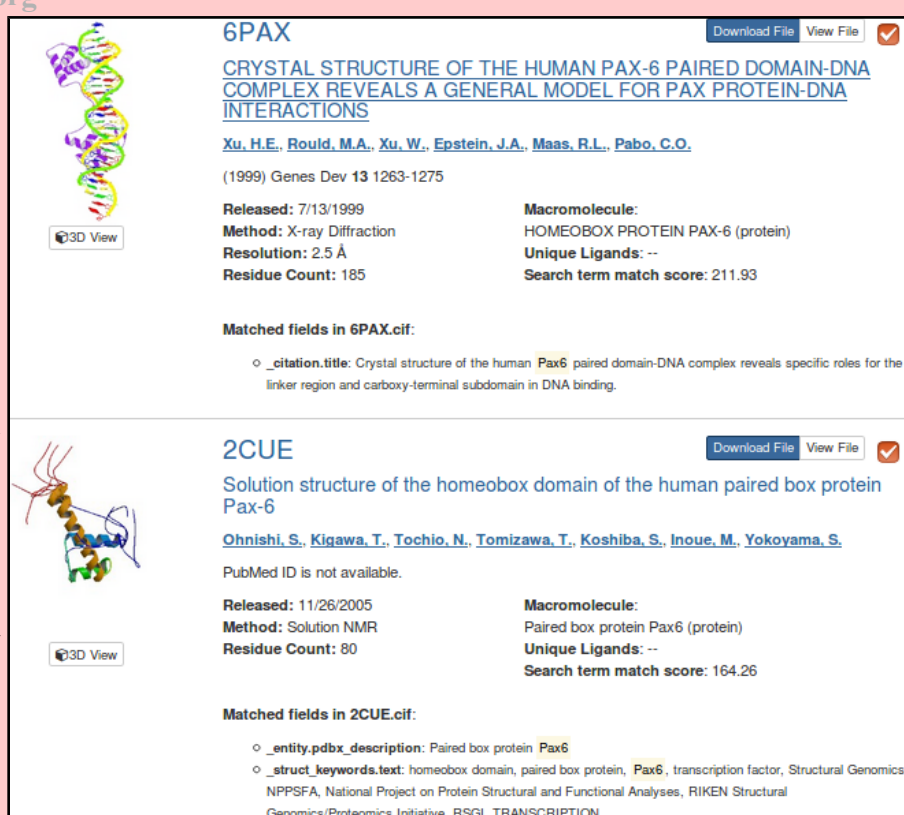
<http://www.rcsb.org>

Enter **PAX6** in the **Search** box and click on the **Go** button.

The two **PDB** structure hits will, hopefully, be familiar. Links are provided with each hit to view the structure with a **3D** viewer , view the textual **PDB** entry or download the **PDB** entry to a file.

Take a look at the **3D** view of the **6PAX** **PDB** entry. This you have seen this previously, but now I suggest a very quick visualisation of the main mutation that causes **Aniridia** occurs in the **PAX6** protein. The idea is to locate and highlight the **Alanine** that mutates to a **Proline** in many **Aniridia** sufferers. As you have discovered, this is the residue **33** in the canonical protein, as recorded by **UniProtKB**. It is residue **30** in the protein as visualised here, the difference being explained by **post translational modification** which, in this instance, removes the first three amino acids. From the **Select a Viewer** menu, choose **JSmol (JavaScript)** as your **3D** viewer.

With your mouse over the structure representation, **Right Click** and select the **Console** option from the menu that will appear.



The screenshot shows the RCSB PDB search results for the query 'PAX6'. Two results are displayed:

- 6PAX**: CRYSTAL STRUCTURE OF THE HUMAN PAX-6 PAIRED DOMAIN-DNA COMPLEX REVEALS A GENERAL MODEL FOR PAX PROTEIN-DNA INTERACTIONS. Released: 7/13/1999. Method: X-ray Diffraction. Resolution: 2.5 Å. Residue Count: 185. Macromolecule: HOMEODOMAIN PROTEIN PAX-6 (protein). Search term match score: 211.93.
- 2CUE**: Solution structure of the homeobox domain of the human paired box protein Pax-6. Released: 11/26/2005. Method: Solution NMR. Residue Count: 80. Macromolecule: Paired box protein Pax6 (protein). Search term match score: 164.26.

Each result includes a 3D view icon and a 'Download File' button.

In the lower text box type in the following commands (there is an extensive manual under the help button if you aspire to be an “expert”):

BACKGROUND BLACK

because I like pictures to have gloomy backgrounds

SELECT 30

to concentrate all further commands just upon the amino acid that varies in many Aniridia patients

SPACEFILL

to make the selected residue stand out

COLOUR CYAN

to make the selected residue stand out even more

Now move the console out of the way and twiddle your structure picture around until you have a good view of the highlighted amino acid and where it lies with respect to the DNA binding helix triplets..

Any comments?



DPJ 2017.12.23

Model Answers to Questions in the Instructions Text.

Notes:

For the most part, these “**Model Answers**” just provide the reactions/solutions I hoped you would work out for yourselves. However, sometime I have tried to offer a bit more back ground and material for thought? Occasionally, I have rambled off into some rather self indulgent investigations that even I would not want to try and justify as pertinent to the objective of these exercises. I like to keep these meanders, as they help and entertain me, but I wish to warn you to only take regard of them if you are feeling particularly strong and have time to burn. Certainly not a good idea to indulge here during a time constrained course event!

Where things have got extreme, I am going to make two versions of the answer. One starting:

Summary:

Which has the answer with only a reasonably digestible volume of deep thought. Read this one.

The other will start:

Full Answer:

Beware of entering here! I do not hold back. Nothing complicated, but it will be long and full of pedantry.

This makes the Model answers section very big. **BUT**, it is not intended for printing or for reading serially, so I submit, being long and wordy does not matter. Feel free to disagree.

From your investigations of Protein Secondary Structure Prediction with Jpred

Some Notes on colouring the MSA generated by Jpred: (Click [here](#) to return to the Instructions.)

Mostly to remind me why and how I decided to colour the **MSA** as I did. My objective was purely to make obvious where the family of proteins were meaningfully similar. If you are happy that this objective was achieved, it is probably best to read no further.

I discovered most of what follows by Selecting the **Help** (easiest way is to press **F1** key, otherwise there is a pull down option at the top of the display, choose **Documentation** option) and searching for “**conservation**”. From the list of hits, I first selected “**Alignment Conservation Annotation**”. There it says:

“**Conservation** is measured as a numerical index reflecting the **conservation** of physico-chemical properties in the alignment: Identities score highest, and the next most conserved group contain substitutions to amino acids lying in the same physico-chemical class.

Conservation is visualised on the alignment or a sequence group as a histogram giving the score for each column. Conserved columns are indicated by ‘*’ (score of 11 with default amino acid property grouping), and columns with mutations where all properties are conserved are marked with a ‘+’ (score of 10, indicating all properties are conserved).

Mousing over a conservation histogram reveals a tooltip which contains a series of symbols corresponding to the physico-chemical properties that are conserved amongst the amino acids observed at each position. In these tooltips, the presence of ! implies that the lack of a particular physico-chemical property is conserved (e.g. !proline).”

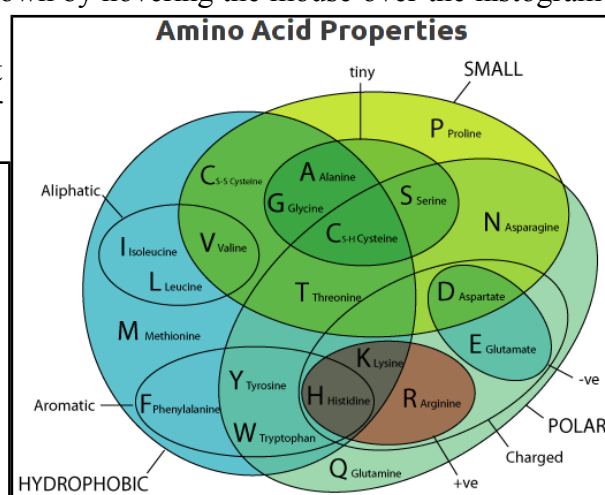
I think to understand the detail of the scoring, one would have to read the paper quoted in the **Help**. I think I will leave that until another day! For now, I just make a few notes.

- The numbers under the histogram columns appear to represent simply the number of physico-chemical properties considered to be conserved. At least, this is consistently true for this example, shown by hovering the mouse over the histogram columns.

- **Jalview** admits to exactly 10 physico-chemical properties that must be one of “**Not conserved**”, “**positively conserved**” or “**negatively conserved**”.

ILVLCAGMFYWHKREQDNSTPBZX-

XXXXXXXXXXXXX.....X...XX	Hydrophobic
.....XXXXXXXXXXXXX.....	Polar
..XXXX.....XXXXX..XX	Small
.....X...XX	Proline
...XX.....X...XX	Tiny
XXX.....XX	Aliphatic
.....XXXX.....XX	Aromatic
.....XXX.....XX	Positive
.....X.X.....XX	Negative
.....XXXX.X.....XX	Charged



- The column achieving a “+” has all 10 conserved physico-chemical properties either **positively** or **negatively conserved**. It is a highly, but not completely conserved “F”. This would appear to agree with the **Help**? There is no example of a 100% conserved column in this example. If there was, I would expect it would be represented by a “*” representing a score of 11.
- Conservation of any given property does not have to be 100% and gaps are tolerated. Reasonable as to be too exacting would eliminating. I expect the details are explained in the original paper. I justify this statement, unnecessarily, by claiming there are both gaps and a **Proline** in the column represented by a “+”.
- I am still uncertain about the difference between a “0” column and a “-” column? I decide to believe they are both columns where there is no measurable conservation, but “0” columns are in regions where they are surrounded by significant conservation? One day, I will read the paper.
- By observation, it can be seen that “conservation” is measured relative to the consensus sequence rather than the query sequence. This seems a reasonable choice to me.

Well that was fun? Now I write some instructions to turn the nasty bland alignment into one that glows blue.

Click [here](#) to return to the Instructions.

What protein database has **Jpred** chosen to search for protein sequences for the alignment upon which its predictions will be based?

The database **Jpred** instructed **PSI-blast** to use to seek proteins homologous to the **PAX6** query can be determined by looking at the sequence identifiers displayed down the left hand side of the alignment in **Jalview**. The identifiers are constructed from the name of the database and the entry identifier separated by an underline character. So the database is the **UniRef90** cluster database built from the **UniProtKB** database.

	110	120	130
QUERY/1-422	EGVCTNDN	IPSVSSINR	VLRLNLA
UniRef90_F6RG82/1-418	EGVCTNDN	IPSVMSMNC	RYRVPR
UniRef90_H2V5M6/1-416	EGVCTNDN	IPSVSSINR	VLRLNDK
UniRef90_B38E4/1-412	EGVCTNDN	IPSVSSINR	VLRLNLA
UniRef90_R0Q084/1-379	EGVCTNDN	IPSVSSINR	VLRLNLA
UniRef90_B7Q450/1-405	EGIVLGAAQ	VSVINR	VLRLNLA
UniRef90_F70053/1-354	DGVCTNDN	IPSVSSINR	VLRLNLA

The **UniRef** cluster databases comprise entries that are not individual protein sequences, but cluster of similar sequences. In the case of the **UniRef90** database, each entry includes all sequences **90%** identical to a given seed sequence. A representative sequence is elected as the only one of the cluster to be considered by such as **PSI-blast**, but clearly, a hit with any representative sequence implies significant similarity with all the sequences of its cluster.

Why do you suppose this database was used in preference to, say **UniprotKB**?

The reason **Jpred** runs **PSI-blast** is to identify sequences representing as wide a family of proteins as possible, to which a **Query** sequence belongs. For the purpose of structure prediction, there is little value in this collection including many sequences that are essentially identical. A wide variety of sequences, as long as they still are likely to be homologous, is of far greater value than a huge number of sequences. Using a **UniRef** database allows that only the **Representative** sequence of each cluster of very similar sequences will be recognised and aligned by **PSI-blast**. This allows the **PSI-blast MSA** to include an extensive range of variation without being bloated by sequences too similar to be individually interesting.

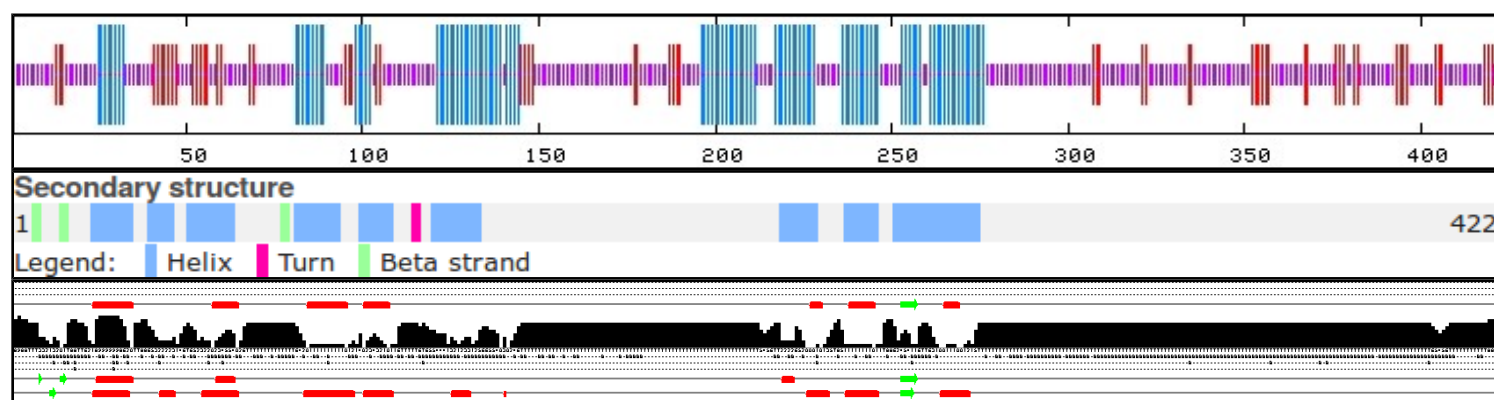
How would you rate the **Jpred** prediction overall?

Well, frankly, not as wonderful as I was expecting. Better than **GOR IV**, but there is still room for improvement? **jnetpred** (essentially the answer) is reasonable. It misses a couple of helices including one that **GOR IV** also overlooks. However, it has considerably less false positive prediction tendencies than **GOR IV**. The **JNETHMM** predictions are particularly poor, saved by the much more accurate deliberations of **JNETPSSM**.

JNETHMM is a prediction computed from the **Hidden Markov Model (HMM)** representation of the final **PSI-blast MSA**.

JNETPSSM is a prediction computed from the **Position Specific Scoring Matrix (PSSM)** representation of the final **PSI-blast MSA**. **PSI-blast** uses **PSSMs** of the **MSA** of each iteration of its search as a **Query** for the next iteration.

The **jnetpred** prediction is effectively the consensus of the predictions of **JNETHMM** and **JNETPSSM**.



Here I have aligned the **GOR IV** and **Jpred** predictions with the secondary structure as recorded by **UniProtKB**.

So, can the prediction be improved? **Jpred** is better than this result suggests!

On reflection, maybe just throwing in the entire sequence of **PAX6_HUMAN** and hoping for the best was a little crude? Our protein has two major domains whose secondary structure one might expect to be conserved. **PSI-blast** will gather together a mountain of sequences that have one, or the other, or both of the domains and try to align them as if they were homologous over their entire length (a **global alignment**). **BUT**, they are not all globally homologous! This means that the alignment of both the domains regions will be polluted by sequence that represent proteins that do not include that domain. This must substantially reduce the quality of the prediction?

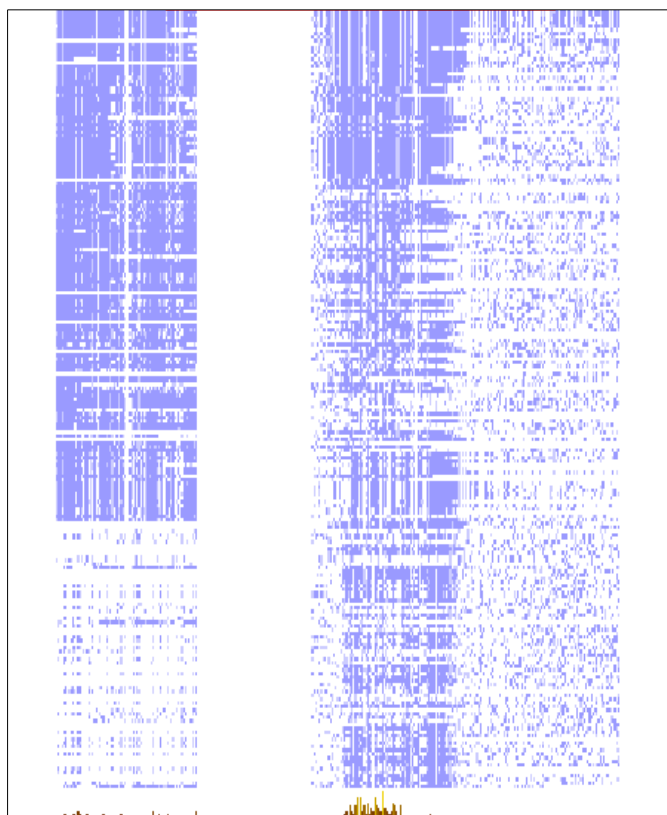
This phenomena can be illustrated by choosing to view the **Jalview Overview Window** (available from the **View** pull down menu)³.

The wider column of blueness at the start of the alignment represents the **paired box** domains. The picture suggests about one third of the aligned sequences do not have a **paired box** domain, but those sequences will have unrelated sequence in that region that will reduce the degree to which the alignment represents the properties of a **paired box** and so also the likelihood of a sensible structure prediction⁴.

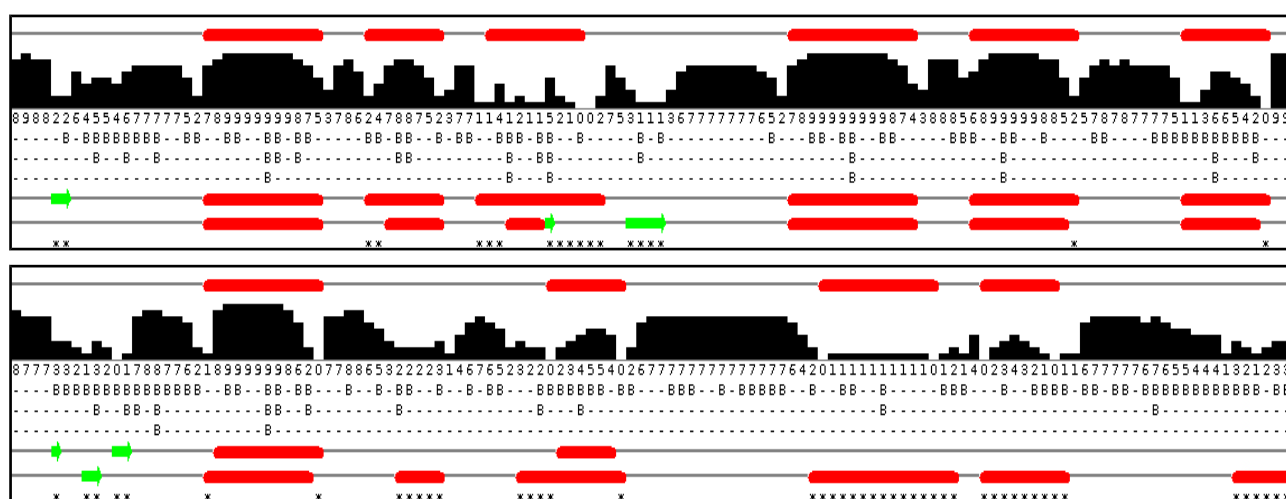
The problem for the more common **homeobox** domain looks less severe, however, the alignment clearly includes many sequences that do not look to have a **homeobox** domain.

So, what to do? I suggest the two domains might be investigated separately? Why not run **Jpred** twice, once with just the **PAX6_HUMAN** paired box region and then again with just the **homeobox** region.

I have done this for you and will now show you the results, however, should you wish to try it yourself, you already have the isolated sequence of both domains saved in local files. The sequence of the **paired box** region should be in a file called **pax_domain.fasta**. The **homeobox** sequence should be in a file called **homeobox_domain.fasta**. Run **Jpred** again with each sequence and you should get results very similar to mine.



First the new **paired box** prediction (top) compared to the original (bottom).

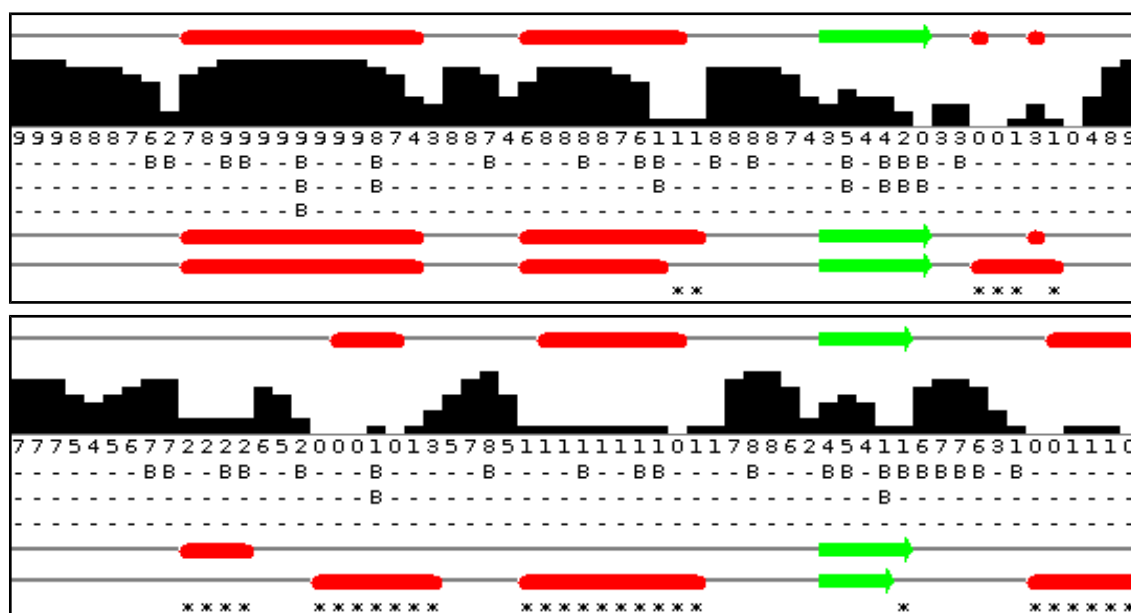


Massively improved I would suggest. All helices present and accurately placed. The **JPREDHMM** prediction, in particular, is very much improved. The **Beta Sheet** predictions seem weak? It finds only one (accurately) of the three that **UniProtKB** suggests to be present. I wonder why, but the helices for the paired box domain specific prediction are excellent.

³ An illustration, in common with all the images presented in this answer, made some time ago, but still reflective of today's results.

⁴ This could be why, as noted in the instructions, the start of the **PAX** region was considered insignificantly conserved by **Jalview**.

And so to the **homeobox** specific results. Once more, the new **homeobox** prediction (top) compared to the original (bottom).



As the **homeobox**s are significantly more numerous than the **paired boxes**, less interference from sequences not including a **homeobox** might have been expected. I imagined the improvement in prediction would be minimal. However, it is very much better! All three helices are predicted in the correct positions, although **Jpred** appears to be a little reluctant about the third helix? There is a rather strong beta sheet prediction that is unsupported by **UniProtKB**. There is no reason to suppose that **UniProtKB** is 100% correct, of course, but nothing I can find suggests that a beta sheet should appear in the middle of a homeobox. An enigma for another day.

So I conclude that this sort of protein analysis requires a little bit more than just throwing an entire sequence at a dumb program and assuming something marvellous will occur. In this case, considering the regions of the protein that are expected to be homologous separately is a very logical thing to do (and entirely obvious, retrospectively at least). Geoff Barton, whose group is responsible for **Jpred** agrees. He says⁵:

“... Always split proteins into domains when searching. ...”

So for both domains the prediction of the helices is far more accurate when each domain is considered separately. However, it is not just the red bars indicating the position of the helical predictions that should be noted. Look also at the confidence histogram. It indicates clearly that with more specific data to work on, better predictions can be made with much improved confidence (i.e. likelihood of being correct!).

DPJ – 2017.12.23

⁵ As does the **Jpred Help** ... and common sense ... I feel a little foolish.

Discussion Points and Casual Questions arising from the Instructions Text.**Notes:****Work in progress I fear.**

The intention is to provide a full consideration of some issues skimmed over in the exercise proper.

If you are attending a “supervised” presentation of the exercise, I would hope to have conducted a live discussion of all these issues to an extent that reflects:

- the depth that seems appropriate
- the time available
- the degree to which the issues seem to match the interests of the class
- how many of you are awake

Here, I hope to write out very full answers were such a response exists. Accordingly, I suggest you will not need to read much of many of these discussions. There will be much detail of interest to rather few of you. Possibly a bit self indulgent, but I wish to make a note of all the background I have discovered while writing these exercises.

In a nutshell, the exercises are trying to make very general points avoiding too much detail. Nevertheless, I record the detail outside the main exercise text, just in case it might be of interest. Some of the answers to the “**Casual Questions**” are exceedingly trivial. Some of the “**Discussion Points**” are exceedingly long and rambling. You have been warned.

A comparative discussion of pHMM and PSSM.

These are both ways to use probabilistic models to represent **Multiple Sequence Alignments (MSAs)**. **PSSMs (Position Specific Scoring Matrices)** are used by **PSI-Blast**, while **pHMMs (profile Hidden Markov Models)** by most of the domain databases we have looked at.

PSSMs and **pHMMs** represent **MSAs** in a similar fashion. That is, both are comprised of sets of likelihood estimates, one for each position of the **MSA**. These represent the relative expectation of each amino acid, a deletion or an insertion, occurring in the corresponding position of a protein that is homologous to the **MSA**.

For **PSSMs**, the likelihoods are computed directly from the **MSA** alone. Thus **PSSMs** are free from the assumption that all evolutionary substitutions must comply with any global model (e.g. the **PAM** or **Blosum** Matrices). For this to work, the **MSA** must be large and representative (as is typical for **PSI-Blast** and similar tools) as they must represent all the evidence for likelihood calculation. **Pseudocounts** (discussed previously) are a way to avoid the consequences of relying on a relative sparse data source, where inadequate depth or exceptionally high conservation could cause misinterpretations.

For **pHMMs**, likelihoods are computed from both the **MSA** and a global model such as represented by the **PAM/Blosum** scoring schemes. As the **MSA** for a **pHMM** is not the sole data source it does not need to be large. It is used only to particularise the message of a global model. For instance, the alignment for the **PAX** domain **pHMM** used by **PFAM** (discussed previously) is comprised of just **5** protein sequences.

A brief consideration of GOR and similar antique secondary structure predictors.

But only brief!!! **GOR** is still available and, presumably, used but is vastly inferior to **Jpred**. Expand later!

Any comment on the highlighting of the PAX6 protein Aniridia mutation position?

Primarily to observe that the mutation is positioned at the end of one of the **Helical Triplets** vital to this proteins **DNA binding** function. It cannot therefore be surprising that it has such profound consequences.

Also, if one was ever to pursue further the examination of **3D structures** in this way, maybe using software that attempts to reflect the consequences of mutations should be considered? Such as **DeepView – Swiss-PdbViewer**, **Maestro** amongst others.

DPJ – 2017.12.23