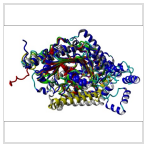

Version 2

Sep 08, 2020

Homology modeling using Phyre2 for Biochemistry I V.2

In 1 collection

Michael Friedman[1], Chris Berndsen[1]

[1]James Madison University

1 *Works for me* This protocol is published without a DOI.

Chris Berndsen
James Madison University

ABSTRACT

Protocol for homology modeling proteins for use in Biochemistry I at James Madison University. Protocol guides students to use the PHYRE2 web server (citation below).

The protocol directs users to save data in OSF or the Open Science Framework. This is the preferred project management tool for the class and is required for JMU students using this for the course. Other users can use whichever system is preferred.

Citations for servers:

1. Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., and Sternberg, M. J. E. (2015) *The Phyre2 web portal for protein modeling, prediction and analysis.* Nat. Protoc. 10, 845–858.

PROTOCOL CITATION

Michael Friedman, Chris Berndsen 2020. Homology modeling using Phyre2 for Biochemistry I. **protocols.io** https://protocols.io/view/homology-modeling-using-phyre2-for-biochemistry-i-bkmfku3n

COLLECTIONS

Biochemistry I methods

KEYWORDS

bioinformatics, phyre2, modeling, protein structure

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PARENT PROTOCOLS

Part of collection
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GUIDELINES

This protocol guides students through homology modeling and analysis of the resulting model. This protocol uses the CRX DNA binding domain to generate the results thus the shown images and results will vary.

The protocol directs users to save data in OSF or the Open Science Framework. This is the preferred project management tool for the class and is required for JMU students using this for the course. Other users can use whichever system is preferred.

MATERIALS TEXT

SWISS-MODEL server: https://swissmodel.expasy.org/

Phyre^2 server: http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index
A sequence in FASTA format
Internet connection
Structure viewing program such as YASARA or UCSF Chimera
Open Science Framework account (JMU students only)

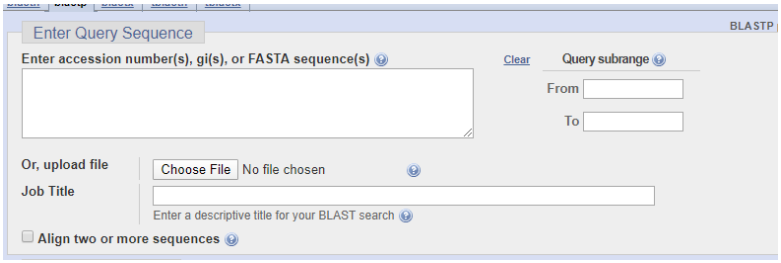
BEFORE STARTING

Gather your sequence in FASTA format (an example is shown below)

```
>seq_name
MASDETEASETEAMDAET
```

NCBI BLAST 10m

1 Navigate to NCBI BLAST (Basic Local Sequence Alignment Tool) and paste your sequence into the "Enter Query Sequence" box.



1.1 The standard settings for the search are shown in the table.

	Default Setting	What it does
Enter Query Sequence		
Query Subrange	*(Blank)*	Limits search to a part of the sequence. Can be useful if there are common motifs/domains in the sequence.
Choose Search Set		

Database	*Non-redundant protein sequences (nr)*	Limits search to a sub-set of sequences. For homology modeling searching the Protein Data Bank proteins (pdb) is a good idea if you want to see if your modeling might be successful.
Organism	*(Blank)*	Limit search to a specific organism or other taxonomic group.
Exclude	*(Unchecked)*	Reduce results by removing certain classifications of sequences.
Program Selection		
Algorithm	*blastp*	Setting changes how the databases are searched. blastp is the most straight-forward. PSI-BLAST is useful when the query sequence is not easily aligned to other sequences.

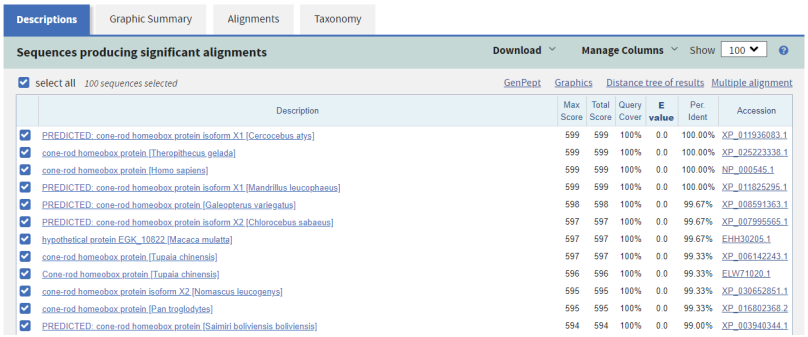
1.2 Record any changes to the settings in Step 2.1 below:

1.3 Press BLAST and wait until the results return.

Thie search can take up to **01:00:00 hour**

Analysis of BLAST results to ID sequence

2 Results will be returned as shown as below:



2.1 Column definitions from the **Descriptions** tab of the results.

Table column	What it tells you
Description	Tells you identify of matching sequence. Predicted or hypothetical in title indicates protein has not been verified.
Max Score	During alignment identities, similarities, and gaps are scored. This indicates the best score if the sequence was aligned multiple times.
Total Score	If many disconnected parts matched, this is the sum of the max scores for those

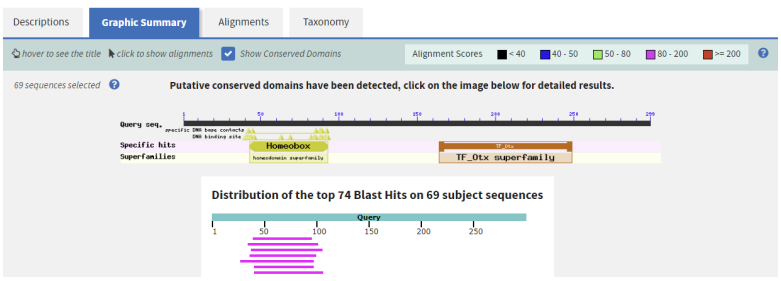
Query Cover	Indicates the percentate of the query sequence found in the match. 100% means all of the sequence was found.
E value	E(xpect) value tells you how many sequences that would rank higher if this was a random match. 0 or very small numbers are good.
Per. Ident	How much of the sequence was identical in sequence. Need >40% for good homology model.
Accession	The accession number for the sequence. Can be clicked to take you to the info card on that sequence.

2.2 Record your best 5 sequences and their statistics in the table below.

Sequence Description	Max Score	Total Score	Query Coverage	E value	Per Ident	Accession

3 In the **Graphic Summary** tab, you can view the domains in your sequence.

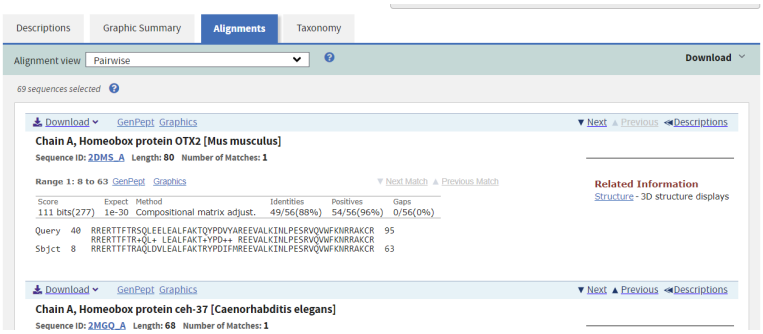
A **domain** is a part of the sequence with a known fold/shape/structure. A **motif** is a sequence that has a shape or function. Typically domains can fold on their on, while motifs are shorter pieces within domains.



3.1 Record any domains or motifs in the table below along with the approximate position within the sequence. This can help in the modeling and support the accuracy of your model later on.

Domain/Motif name	position (this should be a number/set of numbers)

4 In the **Alignments** tab, the actual sequence alignment (the data) are shown.



4.1 Each alignment shows the following key information:

- **Identities** and their location within the sequence.
- **Positives** and their location within the sequence.
- **Gaps** and their location within the sequence.
- **The alignment**: Your sequence is the top row, the matched sequence in the middle row (+ means similar), and the sequece from the database (called Sbjct).
- **Position number** of the sequence match. These are the numbers at each end of the sequences.

4.2 Press the *Download* link to the top right of the alignment and select *Text* you will get a complete file of your results. Upload this to your OSF folder for this project and name the file:

```
BLAST_alignment_[Group_name]_[sequence_name].txt
```

Replace **[Group_name]** with your name/group name without the brackets. Replace **[sequence_name]** with the name of the sequence.

4.3 ⚠

Indicate your OSF file location as a link within a note on this step.

THIS IS YOUR DATA FILE FOR THE SEARCH!

Analysis of BLAST results to ID potential modeling templates

5 and repeat search but limit the Database to Protein Data Bank proteins (pdb). This search will identify proteins of known structure that match your protein and can suggest if your modeling attempt will be successful. Record your sequence matches in the table.

5.1 Accession numbers here lead to the information on the structure which may help when using SWISS-MODEL. These accession numbers are the PDB ID numbers.

Sequence Description	Max Score	Total Score	Query Coverage	E value	Per Ident	Accession

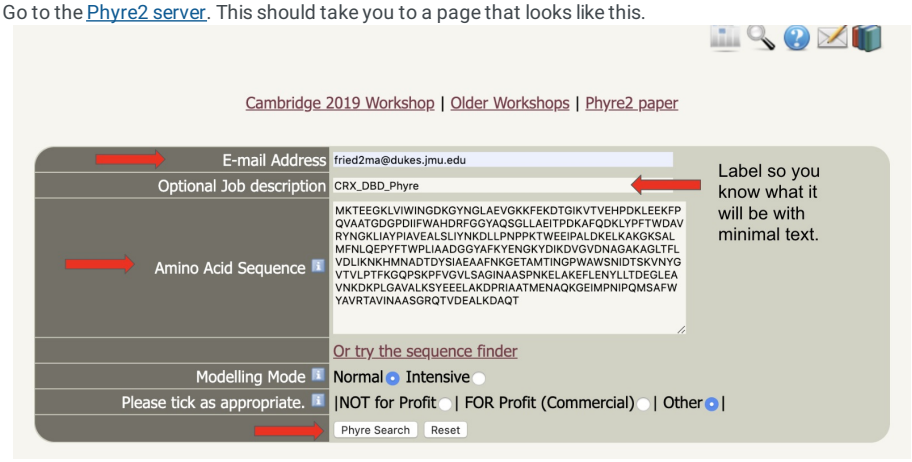
Table for recording results from PDB focused BLAST.

5.2 The top five structures here are potential **templates structures** which you can use to model your sequence. This means these structures are similar at the sequence level to your sequence and *potentially* will result in a similar structure to your sequence.

Homology Modeling

6 Having identified the sequence and potential templates, now it is possible to start modeling the sequence to generate a potential sequence.

7 Go to the Phyre2 server. This should take you to a page that looks like this.



Red arrows indicate the necessary things to change and select.

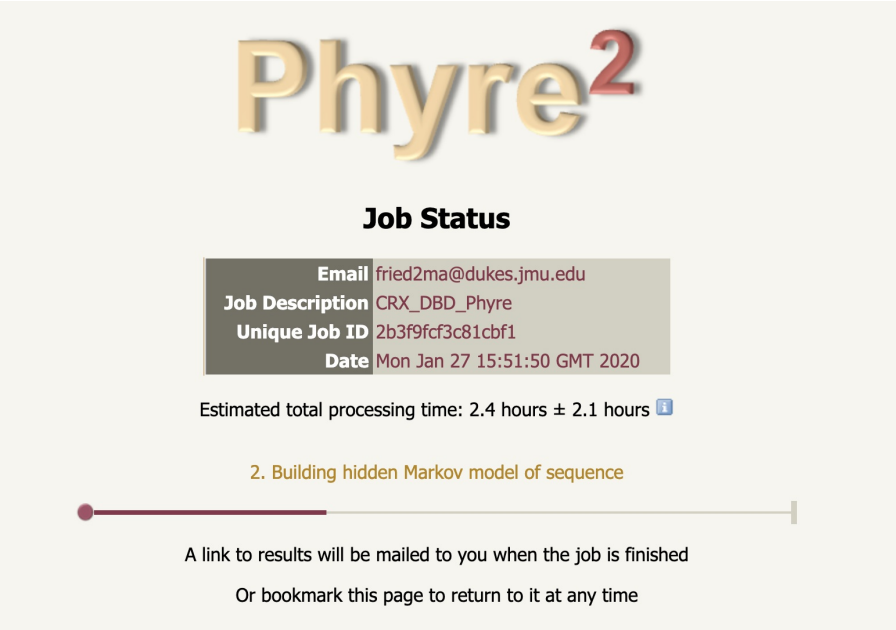
7.1 Paste your sequence into the *Amino Acid Sequence* box as shown above.

7.2 Provide your:

- **email address** so the results and model can be sent to you
- A **job description** so you can keep track of your data
- Which **mode** you want to use. Intensive takes longer but can give better results for models with few templates. Choose normal unless you identified less than 3 templates from BLAST.
- Select **NOT for profit** if you are a JMU student

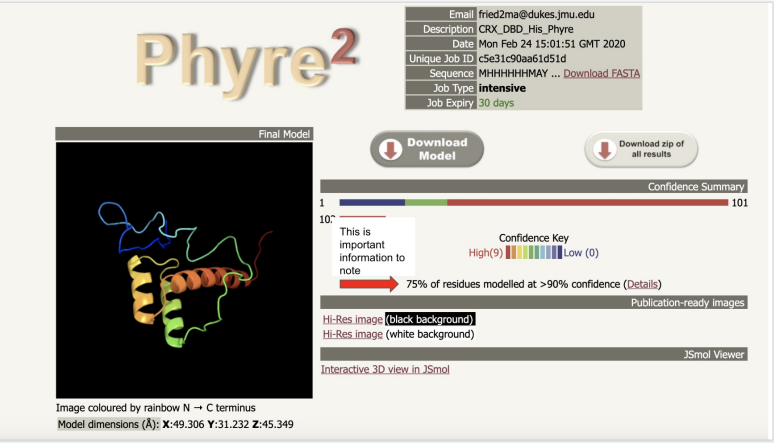
Record your job description in this step as a note.

8 Something like this will appear. Your results will be sent to you via email. Time to retrieve the result varies depending on the server but usually is more than **02:00:00**



Analysis of Phyre2 results

9 Heres a sample of the results linked in the emailed results. Make sure to download this model for compairson. If you find that there are other models that this was built from that you prefer feel free to use their links for compairson too!



9.1 Note the percentage of residues modelled and the location of low confidence regions from the scheme in the **Confidence Summary** box.

Percent of residues modeled:	
Low confidence region locations:	

9.2 Download the model and the zip of all results and upload these files into OSF.

Name the .pdb file as:

```
PHYRE_model_[Group_name]_[sequence_name].pdb
```

Replace **[Group_name]** with your name/group name without the brackets. Replace

[sequence_name] with the name of the sequence.

Name the .zip file as:

```
PHYRE_results[Group_name]_[sequence_name].zip
```

Replace **[Group_name]** with your name/group name without the brackets. Replace [sequence_name] with the name of the sequence.

9.3 Indicate your OSF file location as a link within a note on this step.

THIS IS YOUR DATA FILE FOR THE PHYRE modeling!

10 In the **Sequence analysis** section, you can download the sequence alignment file used in the modeling.

11 The **Secondary structure and disorder prediction** section, you can see what the predicted secondary structure is along with the confidence in that prediction (9 is high, 0 is low). Also, the disorder prediction is shown with ? suggesting disorder and the confidence in that prediction (9 is high, 0 is low).

A PDF of this figure can be download using the symbol on the left.

11.1 Upload your PDF to OSF.

Name the .pdf file as:

```
PHYRE_SecStrPred_[Group_name]_[sequence_name].pdf
```

Replace **[Group_name]** with your name/group name without the brackets. Replace **[sequence_name]** with the name of the sequence.

11.2 Indicate your OSF file location as a link within a note on this step.

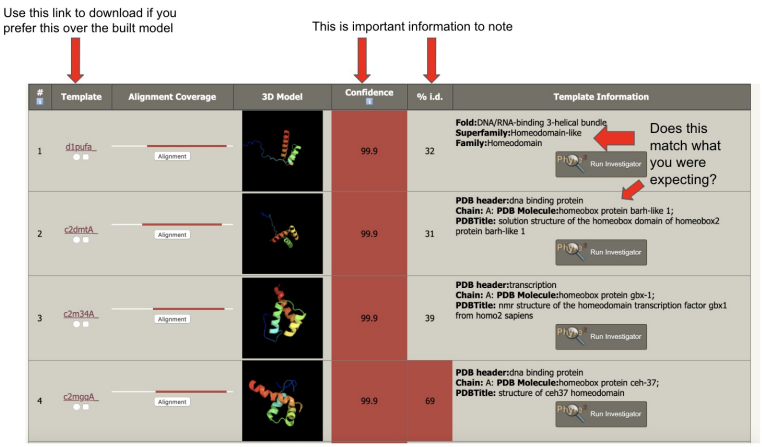
12 In the **Domain Analysis** section, you can move the cursor over each red part of the aligned region and see the predicted domains.

This should match the domains identified in step 3!

12.1 Record the code and the domain name for the top 5 hits in the table.

Code	Domain/motif

13 In the **Detailed Template information** table, there is important information about the templates.



13.1 Take a screen shot of the table showing the top 5 hits and upload the photo to OSF.

Name the file as:

```
PHYRE_templateinfo_[Group_name]_[sequence_name]
```

Replace **[Group_name]** with your name/group name without the brackets. Replace **[sequence_name]** with the name of the sequence.

13.2 Indicate your OSF file location as a link within a note on this step.

14 Save your record, export it as a PDF, and place it in the OSF folder for your notebook files. *If this is part of the modeling project, make sure that you also modeled using SWISS-model*