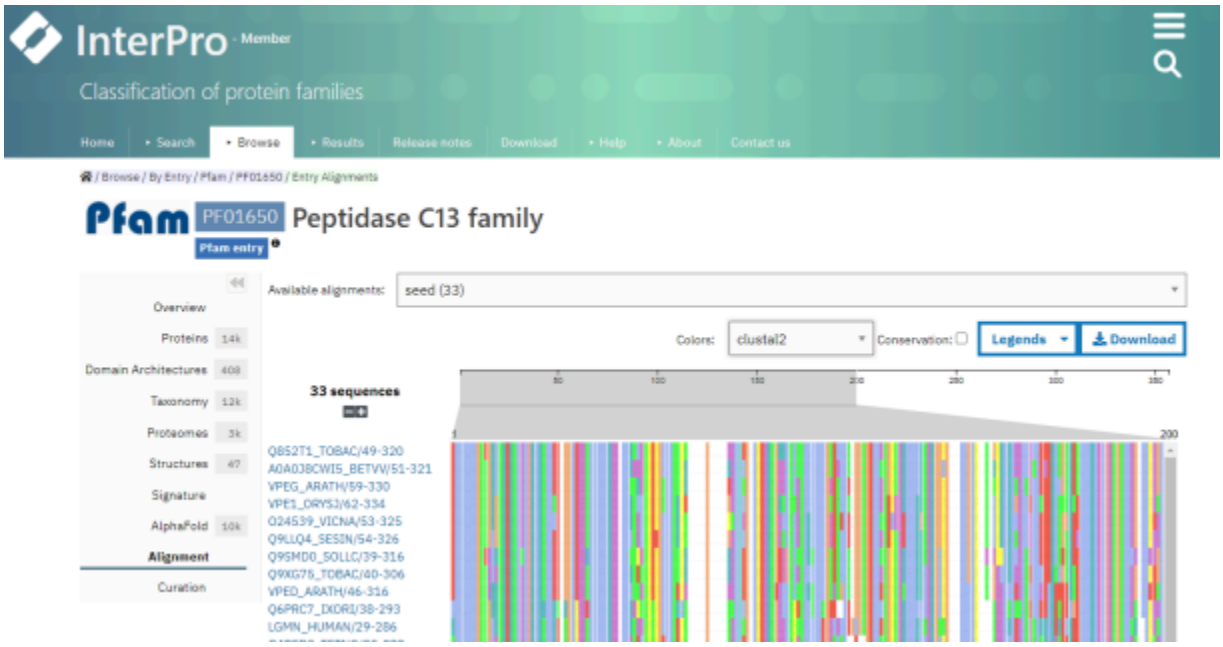
Disclaimer: complete accuracy is not guaranteed.

1. Collect Pfam “seed” entry:

Select the Pfam entry of your protein of interest (POI). Go to the “Alignment” tab. From “Available alignments” select “seed”. Download the seed alignment in clustal2 format. For this tutorial, Peptidase C13 was used as an example POI.

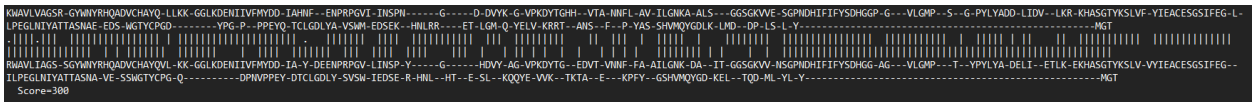


2. Use the “Visual” pairwise sequencing code:

To visualize the pairwise sequence alignment between 2 homologous sequences of the POI, input the stockholm file from Pfam into the “pairwise_tool.py” module (a biopython code using the AlignIO and pairwise 2 packages):

```python
from Bio import AlignIO, pairwise2

sto_file = r"C:\Users\Tania\Downloads\PF01650.alignment.seed\PF01650.alignment.seed" #Insert your .seed stockholm/clustal2 filepath here

alignment = AlignIO.read(r"C:\Users\Tania\Downloads\PF01650.alignment.seed\PF01650.alignment.seed", "stockholm") #Insert your .seed stockholm/clustal2 filepath again

seq1 = alignment[0].seq
seq2 = alignment[1].seq

alignments = pairwise2.align.globalxx(seq1, seq2)

for alignment in alignments:
    print(pairwise2.format_alignment(*alignment))
```

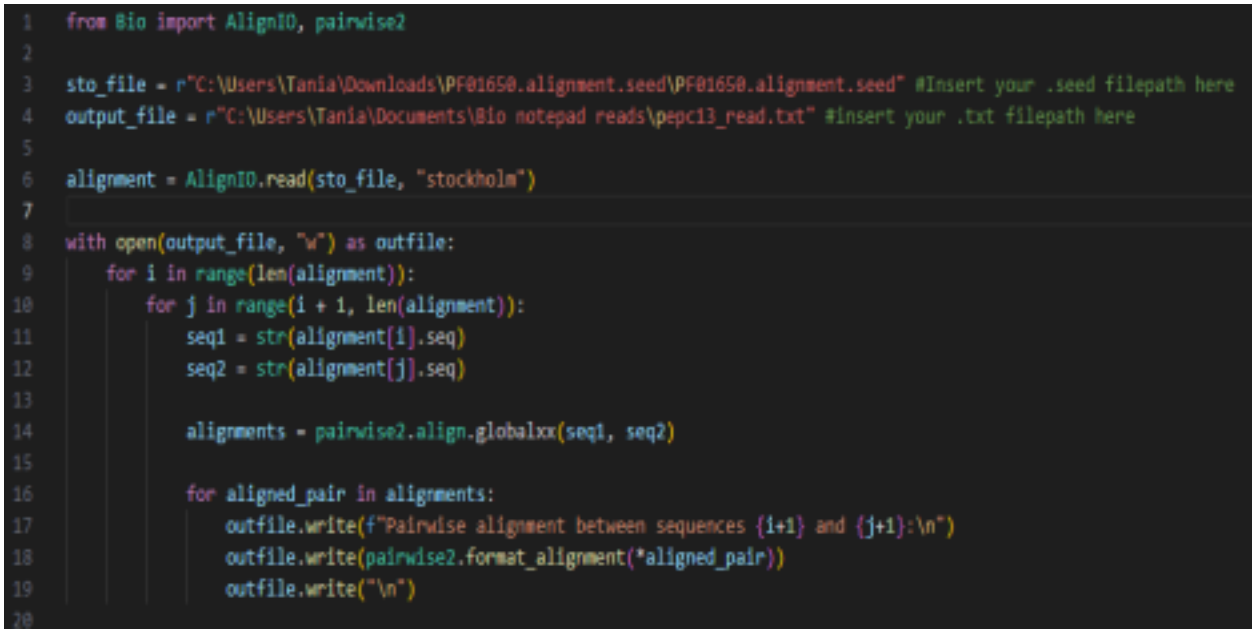
You should receive an output like this (Note: the bottom alignment may appear “slanted”):



A match score can be found below the alignment.

The Pairwise ALL tool can be used to create a pairwise visualization and find a match score between every sequence at once.

WARNING AND DISCLAIMER, PLEASE READ: You can use the "pairwise_tool_ALL_seq.py" module to conduct a sequence alignment between each and every sequence in the SEED file; however, this will take up a lot of memory, a lot of computer processing power, and it may even crash your computer. Running the code on VS studio may give you a bunch of random numbers in the terminal. You can simply exit VS studio, and you should still have some, if not all pairwise sequences, analyzed on the output .txt file.



```python
from Bio import AlignIO, pairwise2

sto_file = r"C:\Users\Tania\Downloads\PF01650.alignment.seed\PF01650.alignment.seed" #Insert your .seed filepath here
output_file = r"C:\Users\Tania\Documents\Bio notepad reads\pepc13_read.txt" #insert your .txt filepath here

alignment = AlignIO.read(sto_file, "stockholm")

with open(output_file, "w") as outfile:
    for i in range(len(alignment)):
        for j in range(i + 1, len(alignment)):
            seq1 = str(alignment[i].seq)
            seq2 = str(alignment[j].seq)

            alignments = pairwise2.align.globalxx(seq1, seq2)

            for aligned_pair in alignments:
                outfile.write(f"Pairwise alignment between sequences {i+1} and {j+1}:\n")
                outfile.write(pairwise2.format_alignment(*aligned_pair))
                outfile.write("\n")
```