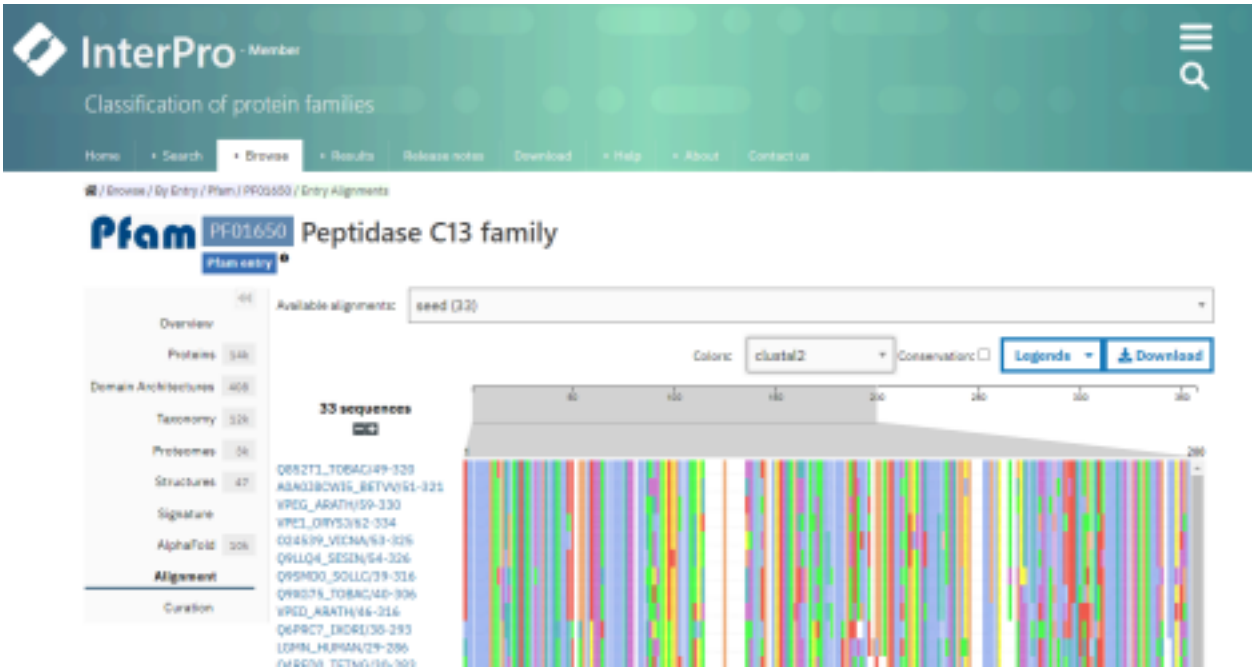
Disclaimer: complete accuracy is not guaranteed.

1. Collect Pfam “seed” entry:

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



2. Use the “Visual” sequence specific selection pairwise sequencing code:

To find the pairwise sequence alignment between 2 homologous sequences of the neurotransmitter membrane channel, a stockholm file from Pfam was input into a biopython code using the pairwise 2 package. This step was repeated with 2 homologous sequences of the protein of interest: Peptidase C13. This was also done using a stockholm file of the protein of interest found on Pfam. This was done using the code below:

```
from Bio import AlignIO, pairwise2

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

alignment = AlignIO.read(r"C:\Users\fanla\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:

Complete pairwise sequencing code:

WARNING, PLEASE READ: You can use the code below 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")

```