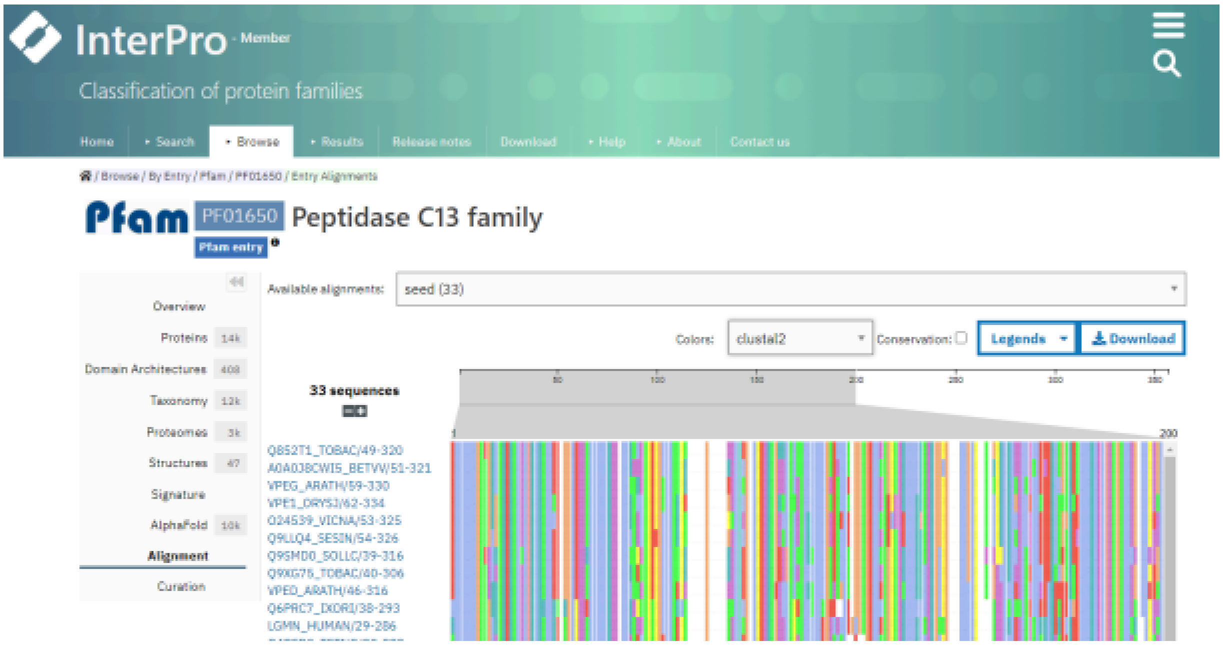
1. Collect Pfam "seed" entry:

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 code below (a biopython code using the pairwise 2 package):

```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:

```
KWAVLVAGSR-GYWNYRHQADVCHAYQ-LLKK-GGLKDENIIVFMYDD-IAHNF--ENPRPGVI-INSPN------G-----D-DVYK-G-VPKDYTGHH--VTA-NNFL-AV-ILGNKA-ALS---GGSGKVVE-SGPNDHIFIFYSDHGGP-G---VLGMP--S--G-PYLYADD-LIDV--LKR-KHASGTYKSLVF-YIEACESGSIFEG-L-
LPEGLNIYATTASNAE-EDS-WGTYCPGD--------YPG-P--PPEYQ-TCLGDLYA-VSWM-EDSEK--HNLRR----ET-LGM-Q-YELV-KRRT--ANS--F--P-YAS-SHVMQYGDLK-LMD--DP-LS-L-Y---------------------------------------------------MGT
RWAVLIAGS-SGYWNYRHQADVCHAYQVL-KK-GGLKDENIIVFMYDD-IA-Y-DEENPRPGV-LINSP-Y-----G------HDVY-AG-VPKDYTG--EDVT-VNNF-FA-AILGNK-DA--IT-GGSGKVV-NSGPNDHIFIFYSDHGG-AG---VLGMP---T--YPYLYA-DELI--ETLK-EKHASGTYKSLV-VYIEACESGSIFEG--
ILPEGLNIYATTASNA-VE-SSWGTYCPG-Q---------DPNVPPEY-DTCLGDLY-SVSW-IEDSE-R-HNL--HT--E-SL--KQQYE-VVK--TKTA--E---KPFY--GSHVMQYGD-KEL--TQD-ML-YL-Y---------------------------------------------------MGT
  Score=300
```

FULL pairwise sequencing code (PLEASE READ WARNING):

WARNING, PLEASE READ: You can use the code below to conduct a sequence alignment between each 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 terminal. Running the code on VS studio may give you a bunch of random numbers in the terminal. Using an output file is strongly recommended for this program. If the program crashes, you can 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")

```