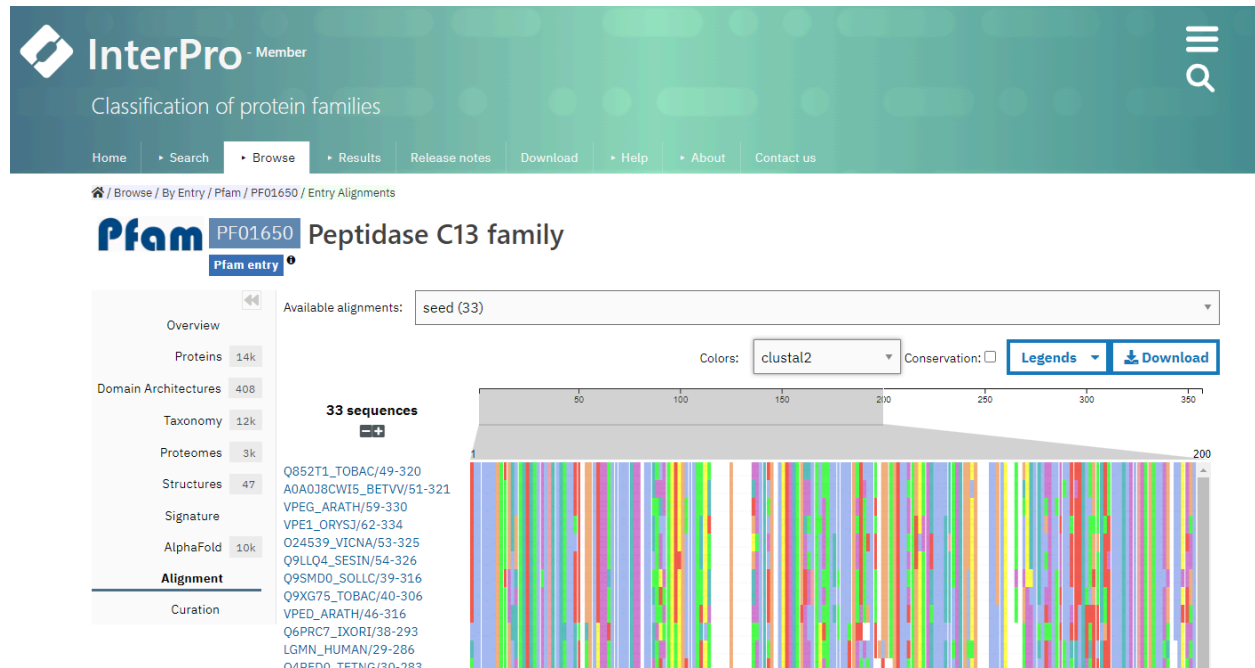


## 1. Collect Pfam “seed” entry:

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.



InterPro - Member  
Classification of protein families

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**Pfam** PF01650 Peptidase C13 family  
Pfam entry

Overview  
Proteins 14k  
Domain Architectures 408  
Taxonomy 12k  
Proteomes 3k  
Structures 47  
Signature  
AlphaFold 10k  
**Alignment**  
Curation

Available alignments: seed (33)

Colors: clustal2 Conservation: ☐ Legends Download

33 sequences

Q852T1\_TOBAC/49-320  
A0A038CWI5\_BETVV/51-321  
VPEG\_ARATH/59-330  
VPE1\_ORYSJ/62-334  
O24539\_VICNA/53-325  
Q9LLQ4\_SESIN/54-326  
Q9SMD0\_SOLLC/39-316  
Q9XG75\_TOBAC/40-306  
VPED\_ARATH/46-316  
Q6PRC7\_DXORI/38-293  
LGMN\_HUMAN/29-286  
U1RFD0\_TETNG/30-283

## 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 pairwise2 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:

```
1 from Bio import AlignIO, pairwise2
2
3 sto_file = r"C:\Users\Tania\Downloads\PF01650.alignment.seed\PF01650.alignment.seed" #Insert your .seed stockholm/clustal2 filepath here
4
5 alignment = AlignIO.read(r"C:\Users\Tania\Downloads\PF01650.alignment.seed\PF01650.alignment.seed", "stockholm") #Insert your .seed stockholm/clustal2 filepath again
6
7 seq1 = alignment[0].seq
8 seq2 = alignment[1].seq
9
10 alignments = pairwise2.align.globalxx(seq1, seq2)
11
12 for alignment in alignments:
13     print(pairwise2.format_alignment(*alignment))
```

You should receive an output like this:

```
KNAVLVAGSR-GYMYRHRQDVCHAYQ-LKK-GGLKDNITVPMYDD-IAHIF--ENRPRQVI-INSPN-----G----D-DVYK-G-VPKDYTGHH-VTA-INPL-AV-ILQNK-ALS---GSGSKVE-SGPNHIFIFYSOHGGP-G--VLGMP--S--G-PVLYADD-LIDV--LKR-KHASGTYSKLVF-YIEACESGSIFEG-L-
LPEGLNIYATTASNA-EES-IGTKCPGQ-----YPG-P--PPQYQ-TCLGDLYA-VSMH-EDSEK--HILRR----ET-LGM-Q-YELV-KRRT--ANS--F--P-YAS-SHMYGDLX-LVD--DP-LS-L-Y-----MET
|||||
RWAVLTAGS-SGYMYRHRQDVCHAYQVL-KK-GGLKDNITVPMYDD-IA-Y-DEENPRPGV-LTNSP-Y-----G-----HDVY-AG-VPKDYTG--EDVT-VNMF-FA-AILQNK-DA--IT-GSGSKV-VNSGPNHIFIFYSOHGG-AG--VLGMP--T--YPVLYA-DELT--ETLK-EKHASGTYSKLV-VYIEACESGSIFEG--
ILPEGLNIYATTASNA-VE-SSMGTCYCPG-Q-----DPMVPEY-DTCLGDLY-SVSM-IEDSE-R-HNL--HT--E-SL--KQYQ-VVK--TKTA-E--KPFY--GSHVQVGD-KEL--TQD-NL-YL-Y-----MGT
Score=300
```

### Complete pairwise sequencing code:

**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 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.

```
1  from Bio import AlignIO, pairwise2
2
3  sto_file = r"C:\Users\Tania\Downloads\PF01650.alignment.seed\PF01650.alignment.seed" #Insert your .seed filepath here
4  output_file = r"C:\Users\Tania\Documents\Bio notepad reads\pepc13_read.txt" #insert your .txt filepath here
5
6  alignment = AlignIO.read(sto_file, "stockholm")
7
8  with open(output_file, "w") as outfile:
9      for i in range(len(alignment)):
10         for j in range(i + 1, len(alignment)):
11             seq1 = str(alignment[i].seq)
12             seq2 = str(alignment[j].seq)
13
14             alignments = pairwise2.align.globalxx(seq1, seq2)
15
16             for aligned_pair in alignments:
17                 outfile.write(f"Pairwise alignment between sequences {i+1} and {j+1}:\n")
18                 outfile.write(pairwise2.format_alignment(*aligned_pair))
19                 outfile.write("\n")
20
```