**Choosing species to run ClustalOmega – 3 methods**

Method 1 – BLAST the S. cerevisiae protein in Uniprot & choose every n-th species to download & use for the alignment (maybe every 10th? – for 20 or so sequences total?)

This is what you had a question in Discord about – I am hesitant to have you discard the entries that don’t have the original name, because sometimes the protein name changes between different species. For now, don’t discard any & choose every 10th one, for example, to do the alignment.

Method 2 – Look up the protein for all the fungal species below & use for alignment; Each can be searched directly in Uniprot and sequence downloaded

Schizosaccharomyces pombe

Scheffersomyces stipitis

Ogataea parapolymorpha

Komagataella phaffii

Cyberlindnera fabianii

Kluyveromyces lactis

Vanderwaltozyma polyspora

Zygosaccharomyces rouxii

Saccharomyces paradoxus

Candida glabrata

Lachancea fermentati

Ashbya gossypii

Torulaspora delbruecki

Kluyveromyces lactis

Naumovozyma castellii

Cyberlindnera jadinii

Pachysolen tannophilus

Candida auris

Method 3 - Look up the protein for the species below (from yeast to human) & use for alignment; Each can be searched directly in Uniprot and sequence downloaded, but you may run into issues if the protein name is not the same in higher organisms as it is in yeast

Saccharomyces cerevisiae

Drosophila melanogaster

Danio reiro

Xenopus laevus

Gallus gallus

Mus musculus

Rattus norvegicus

Homo sapiens

**AlphaFold structure prediction**

Go to alphafoldserver.com

Enter protein sequence in the window provided

Once structure has been generated, download the files

Open the five .cif files in ChimeraX (just drag them in to ChimeraX; these are five different structure predictions)

Overlay them by going to Structure Analysis & choosing Matchmaker; just use default settings

Open the Jalview alignment in ChimeraX (just drag it in)

Right click while hovering over the alignment window & choose Structure, then Associations, then choose one of the models (top one is probably fine) – this will associate the alignment with a sequence/structure

Then type into the command line at the bottom: color byattr seq\_conservation palette blue:white:red range -1.5,1.5

Structure should now be colored with red as most conserved & blue as least conserved

Changing the numbers for the range will change the look of the colored conservation

I’m not sure of the “best” numbers to use for a default, but maybe the -1.5 to 1.5 is fine; I’ll get back to you on this

All structures will now be colored by conservation

For easier viewing, go to the Models panel & choose just one of them to be viewed (click off the boxes for the other 4)