## Gene content protocol

Colored letters correspond to the final datasets as described at the end of the protocol.

- A) Create taxa list for analysis. 47 species list created.
- B) Subset 47 species list proteomes for analysis.

Datasets names and taxa number in each:

- **Opi** (47 taxa proteomes)
- **Aco** (44 taxa proteomes)
- **Xen** (41 taxa proteomes)
- **Hol** (47 taxa proteomes 6 Fungi taxa proteomes)
- HolAco (44 taxa proteomes 6 Fungi taxa proteomes)
- **HolXen** (41 taxa proteomes 6 Fungi taxa proteomes)
- **Cho** (47 taxa proteomes 6 Fungi taxa proteomes 2 lchthyosporea/Filasterea taxa proteomes)
- ChoAco (44 taxa proteomes 6 Fungi taxa proteomes 2 Ichthyosporea/Filasterea taxa proteomes)
- ChoXen (41 taxa proteomes 6 Fungi taxa proteomes 2 Ichthyosporea/Filasterea taxa proteomes)
- C) Run DIAMOND with 4 E-values and MCL with 5 I values and create matrices for datasets:

Three datasets from step A (Opi, Aco and Xen). (repeat for homogroups prediction and orthogroups prediction)

D) Run DIAMOND with 1 E-value (1e-3 default) and MCL with 1 I value (1.5 default) and create matrices from datasets:

All datasets from step A.

(repeat for homogroups prediction, suffix "homo" and orthogroups prediction, suffix "ortho")

New matrices are labeled as in step A with additional suffix "disAb" for all and two datasets labeled with additional suffix "Opi":

- Opi-homo/ortho-disAb (47 taxa proteomes)
- **OpiAco-homo/ortho-disAb** (44 taxa proteomes)
- OpiXen-homo-ortho-disAb (41 taxa proteomes)
- Hol-homo/ortho-disAb (47 taxa proteomes 6 Fungi taxa proteomes)
- HolAco-homo/ortho-disAb (44 taxa proteomes 6 Fungi taxa proteomes)
- HolXen-homo/ortho-disAb (41 taxa proteomes 6 Fungi taxa proteomes)
- Cho-homo/ortho-disAb (47 taxa proteomes 6 Fungi taxa proteomes 2 Ichthyosporea/Filasterea taxa proteomes)
- **ChoAco-homo/ortho-disAb** (44 taxa proteomes 6 Fungi taxa proteomes 2 Ichthyosporea/Filasterea taxa proteomes)

- ChoXen-homo/ortho-disAb (41 taxa proteomes 6 Fungi taxa proteomes 2 Ichthyosporea/Filasterea taxa proteomes)
- E) Using TWO matrices created from step D (Opi-homo-disAb and Opi-ortho-disAb) to create new matrices by **Pruning** (method).

New matrices are labeled as in step D but suffix "disAb" changed to suffix "disP":

- No Pruning for the dataset of the 47 taxa proteomes
- **OpiAco-homo/ortho-disP** (44 taxa proteomes)
- OpiXen-homo/ortho-disP (41 taxa proteomes)
- **Hol-homo/ortho-disP** (47 taxa proteomes 6 Fungi taxa proteomes)
- HolAco-homo/ortho-disP (44 taxa proteomes 6 Fungi taxa proteomes)
- HolXen-homo/ortho-disP (41 taxa proteomes 6 Fungi taxa proteomes)
- **Cho-homo/ortho-disP** (47 taxa proteomes 6 Fungi taxa proteomes 2 Ichthyosporea/Filasterea taxa proteomes)
- **ChoAco-homo/ortho-disP** (44 taxa proteomes 6 Fungi taxa proteomes 2 Ichthyosporea/Filasterea taxa proteomes)
- **ChoXen-homo/ortho-disP** (41 taxa proteomes 6 Fungi taxa proteomes 2 Ichthyosporea/Filasterea taxa proteomes)
- F) Reduce from all datasets in A three ingroup taxa.
- G) Repeat step D for all new datasets from F.

New matrices are labeled as in step D but suffix "disAb" changed to suffix "neAb":

- Opi-homo/ortho-neAb (47 taxa proteomes 3 ingroups taxa proteomes)
- **OpiAco-homo/ortho-neAb** (44 taxa proteomes 3 ingroups taxa proteomes)
- OpiXen-homo-ortho-neAb (41 taxa proteomes 3 ingroups taxa proteomes)
- Hol-homo/ortho-neAb (47 taxa proteomes 6 Fungi taxa proteomes 3 ingroups taxa proteomes)
- **HolAco-homo/ortho-neAb** ( 44 taxa proteomes 6 Fungi taxa proteomes 3 ingroups taxa proteomes)
- HolXen-homo/ortho-neAb (41 taxa proteomes 6 Fungi taxa proteomes 3 ingroups taxa proteomes)
- **Cho-homo/ortho-neAb** (47 taxa proteomes 6 Fungi taxa proteomes 2 Ichthyosporea/Filasterea taxa proteomes 3 ingroups taxa proteomes)
- ChoAco-homo/ortho-neAb (44 taxa proteomes 6 Fungi taxa proteomes 2 Ichthyosporea/Filasterea taxa proteomes 3 ingroups taxa proteomes)
- **ChoXen-homo/ortho-neAb** (41 taxa proteomes 6 Fungi taxa proteomes 2 lchthyosporea/Filasterea taxa proteomes 3 ingroups taxa proteomes)
- H) Repeat Pruning of three ingroup species (same species as in step F), using TWO matrices created from step E (Opi-homo-disP and Opi-ortho-disP) to create new matrices.

New matrices are labeled as in step E but suffix "disP" changed to suffix "neP":

- **Opi-homo/ortho-neP** (47 taxa proteomes - 3 ingroups taxa proteomes)

- **OpiAco-homo/ortho-neP** (44 taxa proteomes 3 ingroups taxa proteomes)
- **OpiXen-homo/ortho-neP** (41 taxa proteomes 3 ingroups taxa proteomes)
- **Hol-homo/ortho-neP** (47 taxa proteomes 6 Fungi taxa proteomes 3 ingroups taxa proteomes)
- HolAco-homo/ortho-neP ( 44 taxa proteomes 6 Fungi taxa proteomes 3 ingroups taxa proteomes)
- **HolXen-homo/ortho-neP** (41 taxa proteomes 6 Fungi taxa proteomes 3 ingroups taxa proteomes)
- **Cho-homo/ortho-neP** (47 taxa proteomes 6 Fungi taxa proteomes 2 Ichthyosporea/Filasterea taxa proteomes 3 ingroups taxa proteomes)
- **ChoAco-homo/ortho-neP** (44 taxa proteomes 6 Fungi taxa proteomes 2 Ichthyosporea/Filasterea taxa proteomes 3 ingroups taxa proteomes)
- **ChoXen-homo/ortho-neP** (41 taxa proteomes 6 Fungi taxa proteomes 2 Ichthyosporea/Filasterea taxa proteomes 3 ingroups taxa proteomes)
- I) Remove all singletons from datasets.

## Results:

Step C results in 120 Phylogenies.
Steps D and G result in 36 Phylogenies.
Steps E and H result in 34 Phylogenies.
A total of 190 Phylogenies.