MAY 20, 2020

Find Proteins of Unknown Function (PUFs) using Plantannot - Protocol F

Forked from [Find Proteins of Unknown Function \(PUFs\) using Plantannot - Protocol E](#)

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ABSTRACT

OPEN ACCESS

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dx.doi.org/10.17504/protocols.io.bgdkjs4w

External link:
<https://www.machado.cnptia.embrapa.br/plantannot>

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Protocol status: Working
It is working

Created: May 14, 2020

Last Modified: May 20, 2020

PROTOCOL integer ID:
37004

The Plantannot software provides several filters and a text search box that allows searching for molecules by its desired annotation features. These filters are needed to obtain PUFs and to try to relate them to abiotic stress using RNA-seq expression data and co-expression networks. The Filters menu is separated in 8 fields, of those we are going to use only five: "Organism", "Feature type", "Orthology", "Orthologs", and "Coexpression" and

“Analyses”. The “Feature Type” filter has three molecule types, from those the polypeptide box is the only that is going to be always checked and the others blank. By using the other 4 remaining filters, 6 protocols were created as examples of different ways to selecting PUFs. Protocol A: using lack of both homology and protein domain signatures. Protocol B: using lack of homology, presence of domain signatures - trying to select Domains of Unknown Function (DUF) from PFAM, and the text search “Unknown function”. Pr

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Protocol F is intended to find PUFs from organisms that proteins are already public in the NCBI's "nr" database and have no protein domain found by Interproscan. Proteins will be selected using the text search "Unknown function". Also, ortholog groups and co-expression networks will be used to relate proteins to abiotic stress es.

Entering application

- 1 Enter the Plantannot Result's page, with empty filters and text box search: <https://www.machado.cnptia.embrapa.br/plantannot/find/?q=>

Or you can enter the <https://www.machado.cnptia.embrapa.br/plantannot> initial page and click on the magnifying glass with the text box empty as well.

<https://www.machado.cnptia.embrapa.br/plantannot>

Filtering

- 2 Find PUFs from organisms that proteins are already public in the NCBI's "nr" database and have no protein domain found by Interproscan. Proteins will be

selected using the text search "Unknown function". Also, ortholog groups and co-expression networks will be used to relate proteins to abiotic stresses.

Visualize the "Filters" card on the left of the page from step1:

Filters

Organism (53)

apply

☐ *Amaranthus hypochondriacus*
(69,156)

☐ *Amborella trichopoda* (80,538)

☐ *Ananas comosus* (81,072)

☐ *Aquilegia coerulea* (117,123)

Feature type

apply

☐ gene (1,862,010)

☐ mRNA (2,332,974)

☐ polypeptide (2,332,974)

Orthology

apply

☐ no orthology (4,636,180)

☐ orthology (1,891,778)

Coexpression

apply

☐ no co-expression groups
(6,381,557)

☐ co-expression groups (146,401)

Orthologs_coexpression

apply

☐ no co-expression (5,097,464)

☐ co-expression (1,430,494)

Analyses

apply

☐ diamond matches (2,209,087)

☐ interproscan matches (1,903,332)

☐ no diamond matches (4,318,871)

☐ no interproscan matches
(4,624,626)

Biomaterial

apply

☐ Leaf (144,826)

☐ Rosette leaves (21,968)

☐ Seedling (26,971)

Treatment

apply

☐ Dehydration (66,121)

☐ Drought (134,012)

☐ Heat stress (50,409)

☐ Osmotic stress (130,599)

<https://www.machado.cnptia.embrapa.br/plantannot/find/?q=>

- 2.1 In the "Organisms" filter, select any organisms (expand the organism's list using the green arrow) or select all by leaving all boxes empty. We will use *Oropetium thomaeum* as example. Click "apply" to execute the filter:

Organism (53)

apply

☐ *Amaranthus hypochondriacus*
(69,156)

☐ *Amborella trichopoda* (80,538)

☐ *Ananas comosus* (81,072)

☐ *Aquilegia coerulea* (117,123)

☐ *Arabidopsis halleri* (78,830)

☐ *Arabidopsis lyrata* (97,337)

☐ *Arabidopsis thaliana* (98,188)

☐ *Boea hygrometrica* (143,334)

☐ *Boechera stricta* (87,040)

☐ *Brachypodium distachyon*
(140,254)

☐ *Brachypodium stacei* (102,612)

☐ *Brassica oleracea* (106,200)

☐ *Brassica rapa* (127,232)

☐ *Capsella grandiflora* (77,927)

☐ *Capsella rubella* (83,415)

☐ *Carica papaya* (83,355)

☐ *Citrus clementina* (92,391)

☐ *Citrus sinensis* (117,673)

☐ *Cucumis sativus* (82,231)

☐ *Daucus carota* (96,349)

☐ *Eucalyptus grandis* (128,909)

☐ *Eutrema salsugineum* (84,919)

☐ *Fragaria vesca* (98,493)

☐ *Glycine max* (233,338)

☐ *Gossypium raimondii* (192,039)

☐ *Kalanchoe fedtschenkoi* (121,344)

☐ *Kalanchoe laxiflora* (188,815)

☐ *Linum usitatissimum* (130,439)

☐ *Malus domestica* (190,548)

☐ *Manihot esculenta* (115,795)

☐ *Medicago truncatula* (175,532)

☐ *Mimulus guttatus* (95,286)

☐ *Musa acuminata* (109,584)

☒ *Oropetium thomaeum* (85,338)

☐ *Oryza sativa* (147,037)

☐ *Panicum hallii* (136,936)

☐ *Panicum virgatum* (348,885)

☐ *Phaseolus vulgaris* (101,423)

☐ *Populus trichocarpa* (187,361)

☐ *Prunus persica* (121,051)

☐ *Ricinus communis* (93,663)

☐ *Salix purpurea* (160,905)

☐ *Setaria italica* (120,586)

☐ *Setaria viridis* (132,402)

☐ *Solanum lycopersicum* (104,175)

☐ *Solanum tuberosum* (151,458)

☐ *Sorghum bicolor* (128,371)

☐ *Spirodela polyrhiza* (58,869)

☐ *Theobroma cacao* (118,260)

☐ *Trifolium pratense* (122,552)

☐ *Vitis vinifera* (79,038)

☐ *Zea mays* (241,000)

☐ *Zostera marina* (61,350)

https://www.machado.cnptia.embrapa.br/plantannot/find/?q=&selected_facets=organism%3AOropetium+thomaeum

- 2.2 In the "Feature type" filter, select "polypeptide", and click "apply" to execute the filter:

Feature type

apply

remove

☐ gene (0)
☐ mRNA (0)
☒ polypeptide (2,551)

https://www.machado.cnptia.embrapa.br/plantannot/find/?q=&selected_facets=organism%3AOropetium+thomaeum&selected_facets=so_term%3Apolypeptide

- 2.3 In the Orthology filter, select "orthology", and click "apply" to execute the filter:

Orthology

apply

remove

☐ no orthology (0)
☒ orthology (18,755)

https://www.machado.cnptia.embrapa.br/plantannot/find/?q=&selected_facets=organism%3AOropetium+thomaeum&selected_facets=so_term%3Apolypeptide&selected_facets=orthology%3A1

- 2.4 Leave the "Coexpression" filter empty:

Coexpression

apply

☐ no co-expression groups (711)
☐ co-expression groups (0)

- 2.5 In the "Orthologs_coexpression" filter, select "co-expression", and click "apply" to execute the filter:

Orthologs_coexpression

apply

remove

☐ no co-expression (0)
☒ co-expression (15,641)

https://www.machado.cnptia.embrapa.br/plantannot/find/?q=&selected_facets=organism%3AOropetium+thomaeum&selected_facets=so_term%3Apolypeptide&selected_facets=orthology%3A1&selected_facets=orthologs_coexpression%3Atrue

- 2.6 In addition to the applied filters, text search for "Unknown function" in the search box. After entering the text click on the magnifying glass icon to apply the search.

Analyses
apply remove

☒ diamond matches (894)
☐ interproscan matches (0)
☐ no diamond matches (0)
☒ no interproscan matches (894)

https://www.machado.cnptia.embrapa.br/plantannot/find/?q=&selected_facets=organism%3AOropetium+thomaeum&selected_facets=so_term%3Apolypeptide&selected_facets=orthology%3A1&selected_facets=orthologs_coexpression%3Atrue&selected_facets=analyses%3Adiamond+matches&selected_facets=analyses%3Ano+interproscan+matches

2.7 Leave the "Biomaterial" and "Treatment" filters empty:

Biomaterial
apply

☐ Leaf (0)
☐ Rosette leaves (0)
☐ Seedling (0)

Treatment
apply

☐ Dehydration (0)
☐ Drought (0)
☐ Heat stress (0)
☐ Osmotic stress (0)

2.8 In addition to the applied filters, text search for "Unknown function" using the search box. After entering the text click on the magnifying glass icon.

🔍

Filters

Selected filters

Results

Organism	Feature Type	Feature ID	Relationship	Display
<i>Oropetium thomaeum</i>	polypeptide	Protein: 904264AC (1470 A.A.)	DE42992	Protein of unknown function

https://www.machado.cnptia.embrapa.br/plantannot/find/?q=Unknown+function&selected_facets=organism%3AOropetium+thomaeum&selected_facets=so_term%3Apolypeptide&selected_facets=orthology%3A1&selected_facets=orthologs_coexpression%3Atrue&selected_facets=analyses%3Adiamond+matches&selected_facets=analyses%3Ano+interproscan+matches

Filters

3 After execution of all filters we will have the following list of filters:

Selected filters

search:Unknown function ✕
 organism:Oropetium thomaeum ✕
 so_term:polypeptide ✕
 orthology ✕
 coexpression in orthologs ✕
 analyses:diamond matches ✕
 analyses:no interproscan matches ✕

[https://www.machado.cnptia.embrapa.br/plantannot/find/?](https://www.machado.cnptia.embrapa.br/plantannot/find/?q=Unknown+function&selected_facets=organism%3AOropetium+thomaeum&selected_facets=so_term%3Apolypeptide&selected_facets=orthology%3A1&selected_facets=orthologs_coexpression%3Atrue&selected_facets=analyses%3Adiamond+matches&selected_facets=analyses%3Ano+interproscan+matches)

[q=Unknown+function&selected_facets=organism%3AOropetium+thomaeum&selected_facets=so_term%3Apolypeptide&selected_facets=orthology%3A1&selected_facets=orthologs_coexpression%3Atrue&selected_facets=analyses%3Adiamond+matches&selected_facets=analyses%3Ano+interproscan+matches](https://www.machado.cnptia.embrapa.br/plantannot/find/?q=Unknown+function&selected_facets=organism%3AOropetium+thomaeum&selected_facets=so_term%3Apolypeptide&selected_facets=orthology%3A1&selected_facets=orthologs_coexpression%3Atrue&selected_facets=analyses%3Adiamond+matches&selected_facets=analyses%3Ano+interproscan+matches)

Viewing results

- Visualize the "Results" card on the center-right of the screen. There will be the resulting list of *Oropetium*'s PUFs. 4 PUFs were filtered:

Results

Organism	Feature Type	Feature ID	Relationship	Display	Orthologous Group	Coexpression Group
Oropetium thomaeum	polypeptide	Oropetium_20150105_04788A.v1.0	mRNA	PF14365 - Domain of unknown function (DUF4409) (1 of 10)	plantannot2042	
Oropetium thomaeum	polypeptide	Oropetium_20150105_05984A.v1.0	mRNA	PF03478 - Protein of unknown function (DUF295) (DUF295) (1 of 52)	plantannot22362	
Oropetium thomaeum	polypeptide	Oropetium_20150105_05988A.v1.0	mRNA	PF03478 - Protein of unknown function (DUF295) (DUF295) (1 of 52)	plantannot22362	
Oropetium thomaeum	polypeptide	Oropetium_20150105_06185A.v1.0	mRNA	PF04578/PP13968 - Protein of unknown function, DUF594 (DUF594) / Domain of unknown function (DUF4220) (DUF4220) (1 of 12)	plantannot17852	