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

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**Protocol status:** Working

It is working

**Created:** May 13, 2020

**Last Modified:** May 20, 2020

**Protocol Integer ID:** 37004

## Abstract

The Plant annot 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 stresses using RNA-seq expression data and co-expression networks.



The Filter  
s menu  
is separ  
ated in 8  
fields  
, of those  
we are  
going to  
use only  
five:  
"Organism",  
"Feature  
type",  
"Orthology  
"  
"Orthologs  
\_coexpression"  
and  
"Analyses".  
The  
"Feature  
Type"  
filter  
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(DUF) from PFAM, and the text search "Unknown function". Protocol C: using homology, lack of protein domain signatures and the text search "Unknown function". Protocol D-F: same protocols of A-C but using ortholog groups to find homolog proteins with co-expression data related to abiotic stress.



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 tomaeum* 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)

A screenshot of a web interface showing a list of plant species. Each species name is preceded by an unchecked checkbox and followed by a count in parentheses. The species listed are: *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), and *Zostera marina* (61,350). A small green triangle is visible at the bottom center of the list area.

[https://www.machado.cnptia.embrapa.br/plantannot/find/?q=&selected\\_facets=organism%3AOropetium+thomaeum](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:

A screenshot of a web interface showing a filter titled "Feature type". The filter has two buttons: "apply" and "remove". Below the title, there is a list of feature types with checkboxes and counts: "gene (0)", "mRNA (0)", and "polypeptide (2,551)". The "polypeptide" option is selected, indicated by a checked checkbox.

[https://www.machado.cnptia.embrapa.br/plantannot/find/?q=&selected\\_facets=organism%3AOropetium+thomaeum&selected\\_facets=so\\_term%3Apolypeptide](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](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](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

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- 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](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)

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**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.

The screenshot shows a search interface. At the top, there is a search bar containing the text "Unknown function" and a magnifying glass icon circled in red. Below the search bar, there are two main sections: "Filters" and "Results". The "Filters" section shows a "Selected filters" area. The "Results" section displays a table with columns: Organism, Feature Type, Feature ID, Relationship, and Display. The first row of the table shows "Oropetium", "polypeptide", "Oropetium\_20150105\_01079A.v1.0", "mRNA", "BE12022", and "Domain 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=ortho\\_logs\\_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=ortho_logs_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/?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

4 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:

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) (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//PF13968 - Protein of unknown function, DUF594 (DUF594)// Domain of unknown function (DUF4220) (DUF4220) (1 of 12)	plantannot17852	

