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

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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

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Protocol Integer ID: 36981

Abstract

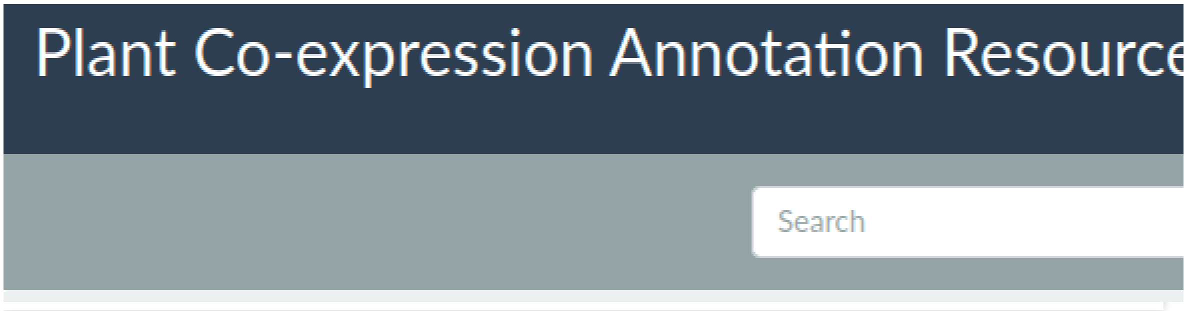
The Plantannot software provides several filters and a text search box that allows searching for molecules by its desired annotation features. These filters are needed to obtain PUFs and to try to relate them to abiotic stresses using RNA-seq expression data and co-expression networks. The Filters menu is separated in 8 fields, of those we are going to use only five: “Organism”, “Feature type”, “Orthology”, “Orthologs_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”.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 A is intended to Find PUFs from organisms whose proteins are not yet in the NCBI´s nr database and have no protein domains found by Interproscan.

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.

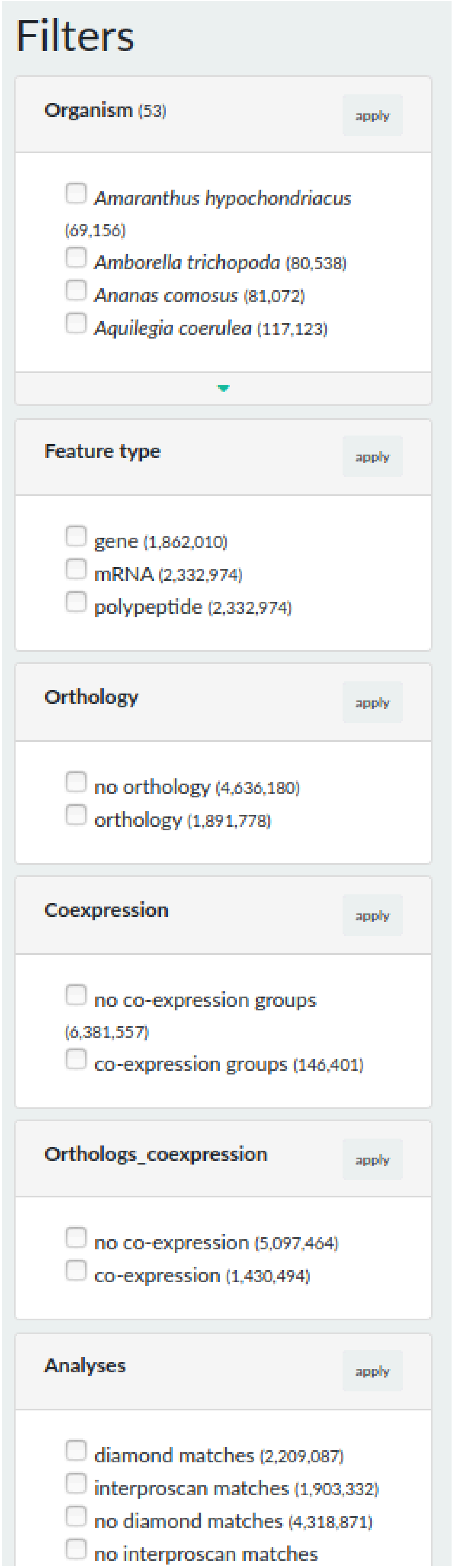


https://www.machado.cnptia.embrapa.br/plantannot

Filtering

2 Find PUFs from organisms whose proteins are not yet in the NCBI´s "nr" database and have no protein domains found by InterproScan.

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



Filters

Organism (53)

☐ *Amaranthus hypochondriacus* (69,156)
☐ *Amborella trichopoda* (80,538)
☐ *Ananas comosus* (81,072)
☐ *Aquilegia coerulea* (117,123)

Feature type

☐ gene (1,862,010)
☐ mRNA (2,332,974)
☐ polypeptide (2,332,974)

Orthology

☐ no orthology (4,636,180)
☐ orthology (1,891,778)

Coexpression

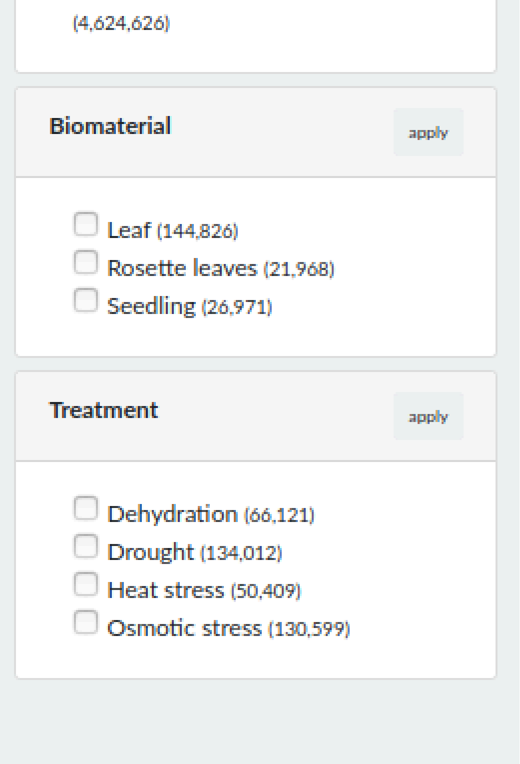
☐ no co-expression groups (6,381,557)
☐ co-expression groups (146,401)

Orthologs_coexpression

☐ no co-expression (5,097,464)
☐ co-expression (1,430,494)

Analyses

☐ diamond matches (2,209,087)
☐ interproscan matches (1,903,332)
☐ no diamond matches (4,318,871)
☐ no interproscan matches



(4,624,626)

Biomaterial apply

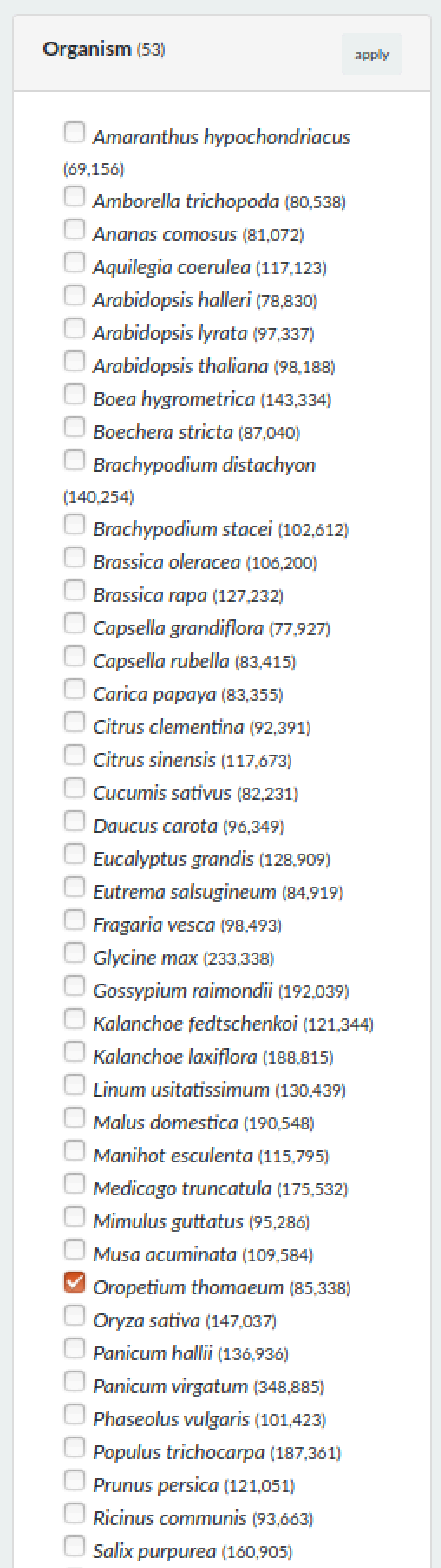
☐ Leaf (144,826)
☐ Rosette leaves (21,968)
☐ Seedling (26,971)

Treatment apply

☐ Dehydration (66,121)
☐ Drought (134,012)
☐ Heat stress (50,409)
☐ Osmotic stress (130,599)

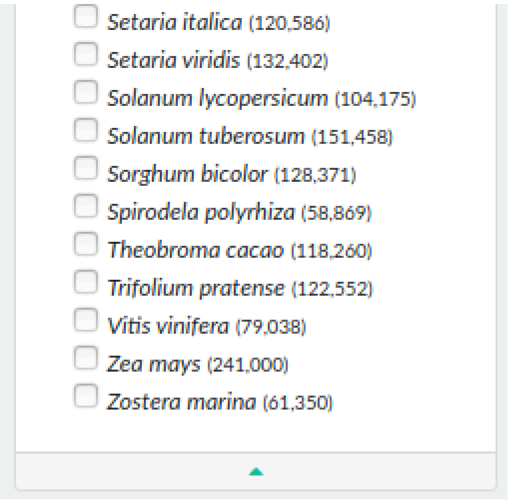
https://www.machado.cnptia.embrapa.br/plantannot/find/?q=

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)

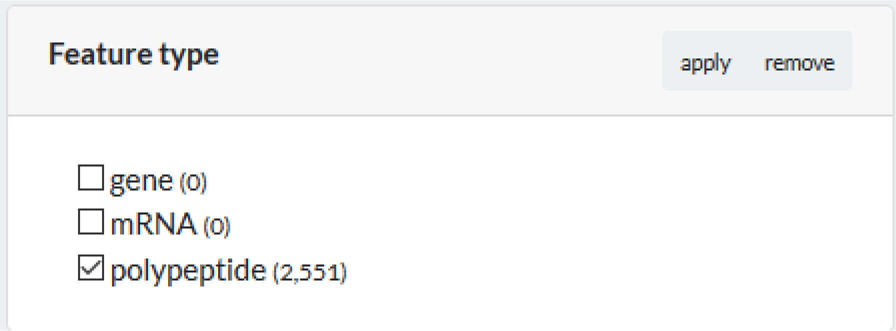
- ☐ *Amaranthus hypochondriacus* (69,156)
- ☐ *Amborella trichopoda* (80,538)
- ☐ *Ananas comosus* (81,072)
- ☐ *Aquilegia coerulea* (117,123)
- ☐ *Arabidopsis halleri* (78,830)
- ☐ *Arabidopsis lyrata* (97,337)
- ☐ *Arabidopsis thaliana* (98,188)
- ☐ *Boea hygrometrica* (143,334)
- ☐ *Boechera stricta* (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:

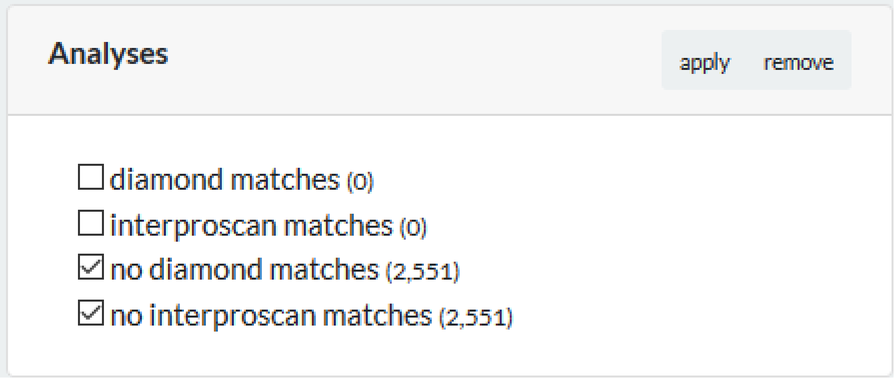


Feature type

☐ 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 "Analyses" filter, select both "no diamond matches" and "no interproscan matches", and click "apply" to execute the filter:

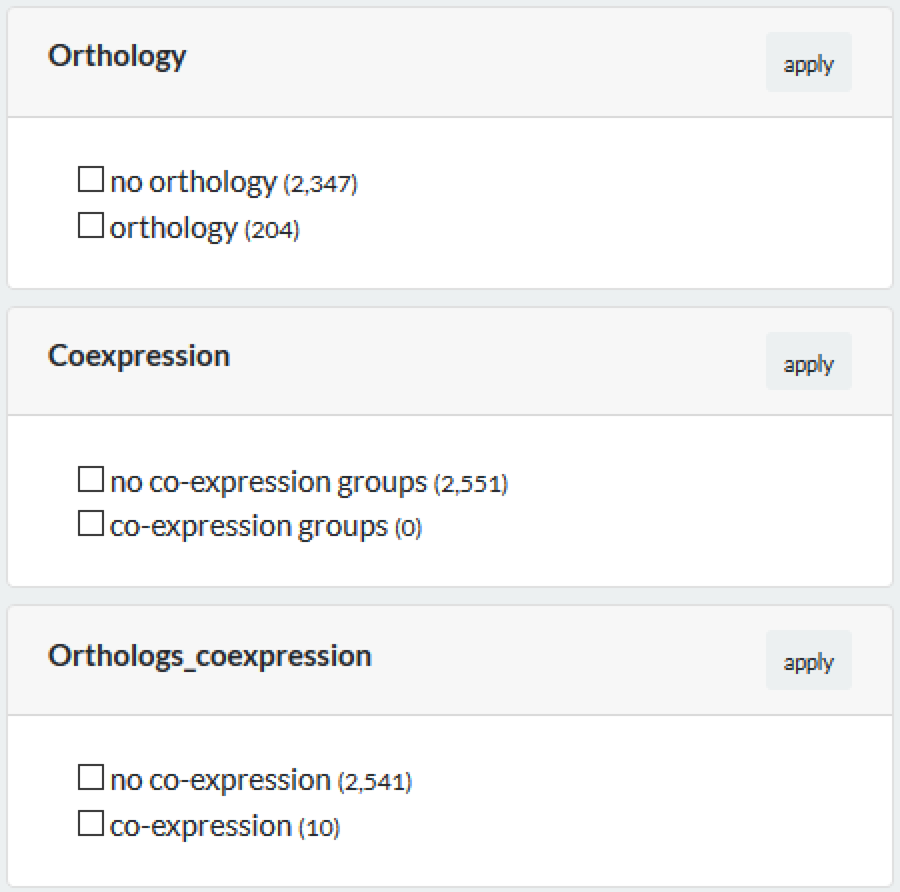


Analyses

☐ diamond matches (0)
☐ interproscan matches (0)
☑ no diamond matches (2,551)
☑ no interproscan matches (2,551)

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

2.4 Leave the "Orthology" and "Coexpression" and "Orthologs_coexpression" filters empty:



Orthology

☐ no orthology (2,347)
☐ orthology (204)

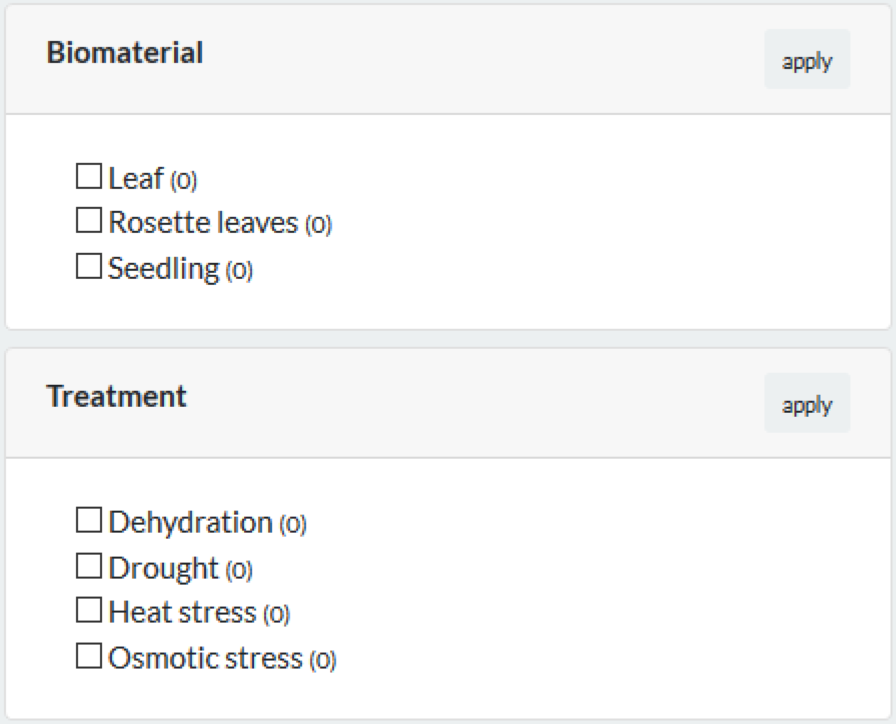
Coexpression

☐ no co-expression groups (2,551)
☐ co-expression groups (0)

Orthologs_coexpression

☐ no co-expression (2,541)
☐ co-expression (10)

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



Biomaterial

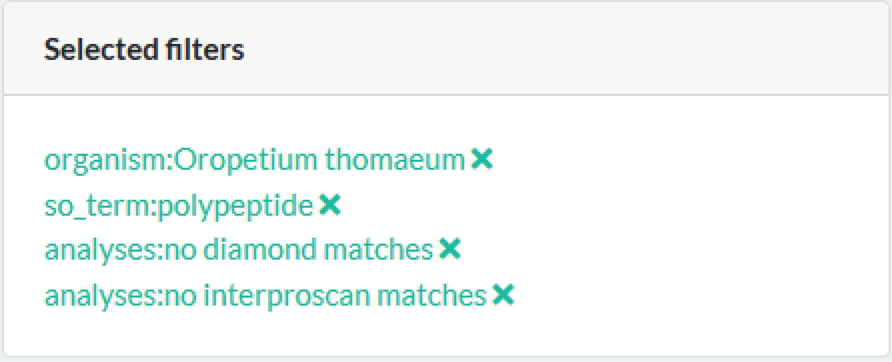
☐ Leaf (0)
☐ Rosette leaves (0)
☐ Seedling (0)

Treatment

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

Filters

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



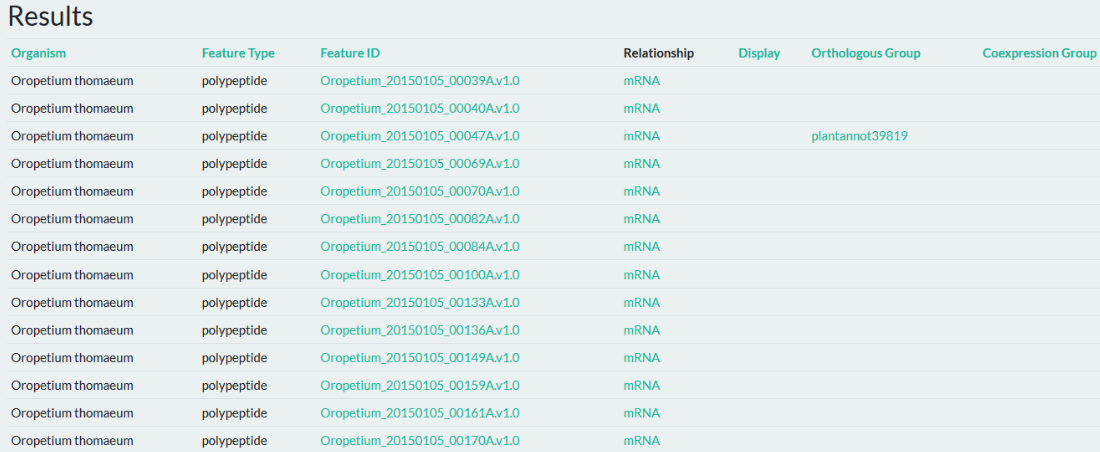
Selected filters

organism:Oropetium thomaeum ✕
so_term:polypeptide ✕
analyses:no diamond matches ✕
analyses:no interproscan matches ✕

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

Viewing results

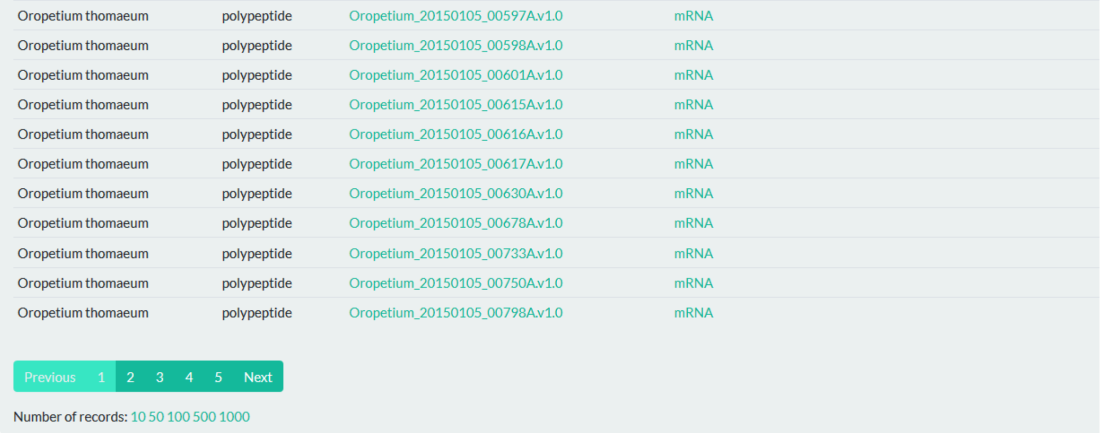
4 Visualize the "Results" card on the center-right of the screen, we will have the resulting list of *Oropetium's* PUFs, 2,541 PUFs were filtered:



Results

Organism	Feature Type	Feature ID	Relationship	Display	Orthologous Group	Coexpression Group
Oropetium thomaeum	polypeptide	Oropetium_20150105_00039A.v1.0	mRNA			
Oropetium thomaeum	polypeptide	Oropetium_20150105_00040A.v1.0	mRNA			
Oropetium thomaeum	polypeptide	Oropetium_20150105_00047A.v1.0	mRNA		plantannot39819	
Oropetium thomaeum	polypeptide	Oropetium_20150105_00069A.v1.0	mRNA			
Oropetium thomaeum	polypeptide	Oropetium_20150105_00070A.v1.0	mRNA			
Oropetium thomaeum	polypeptide	Oropetium_20150105_00082A.v1.0	mRNA			
Oropetium thomaeum	polypeptide	Oropetium_20150105_00084A.v1.0	mRNA			
Oropetium thomaeum	polypeptide	Oropetium_20150105_00100A.v1.0	mRNA			
Oropetium thomaeum	polypeptide	Oropetium_20150105_00133A.v1.0	mRNA			
Oropetium thomaeum	polypeptide	Oropetium_20150105_00136A.v1.0	mRNA			
Oropetium thomaeum	polypeptide	Oropetium_20150105_00149A.v1.0	mRNA			
Oropetium thomaeum	polypeptide	Oropetium_20150105_00159A.v1.0	mRNA			
Oropetium thomaeum	polypeptide	Oropetium_20150105_00161A.v1.0	mRNA			
Oropetium thomaeum	polypeptide	Oropetium_20150105_00170A.v1.0	mRNA			

By default we have 50 results displayed of the screen,but at the bottom of the screen this number can be changed or if you prefer you can borwser of the screens to see all the results.



Oropetium thomaeum	polypeptide	Oropetium_20150105_00597A.v1.0	mRNA
Oropetium thomaeum	polypeptide	Oropetium_20150105_00598A.v1.0	mRNA
Oropetium thomaeum	polypeptide	Oropetium_20150105_00601A.v1.0	mRNA
Oropetium thomaeum	polypeptide	Oropetium_20150105_00615A.v1.0	mRNA
Oropetium thomaeum	polypeptide	Oropetium_20150105_00616A.v1.0	mRNA
Oropetium thomaeum	polypeptide	Oropetium_20150105_00617A.v1.0	mRNA
Oropetium thomaeum	polypeptide	Oropetium_20150105_00630A.v1.0	mRNA
Oropetium thomaeum	polypeptide	Oropetium_20150105_00678A.v1.0	mRNA
Oropetium thomaeum	polypeptide	Oropetium_20150105_00733A.v1.0	mRNA
Oropetium thomaeum	polypeptide	Oropetium_20150105_00750A.v1.0	mRNA
Oropetium thomaeum	polypeptide	Oropetium_20150105_00798A.v1.0	mRNA

Number of records: 10 50 100 500 1000

In addition, at the top right of the results screen you can click on the highlighted icon in the image below and download all the results in a .tsv file

Feature ID	Relationship	Display	Orthologous Group	Coexpression Group