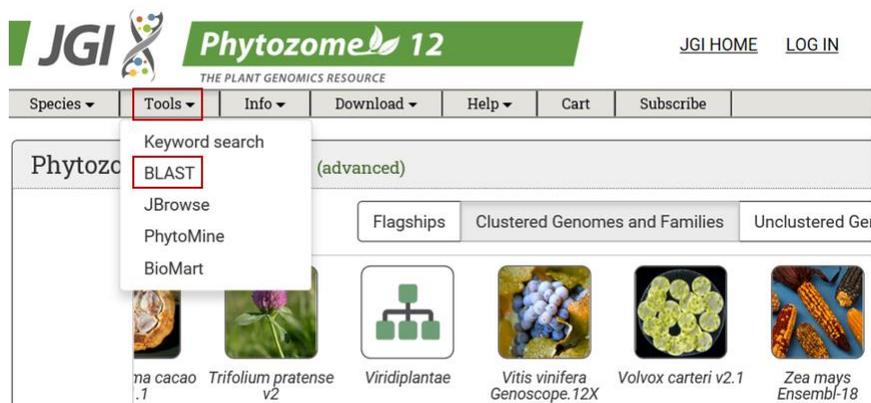


❖ Search for PIN family members from *Marchantia polymorpha*, *Physcomitrella patens*, *Selaginella moellendorffii*, *Capsella rubella*, *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, and *Brachypodium arborescens*.

1. Download the sequence of *Arabidopsis thaliana* PIN1 protein from the TAIR database (<https://www.arabidopsis.org/>).
2. Open the link of Phytozome database (<https://phytozome.jgi.doe.gov/pz/portal.html>),
3. Find the top menu "Tools", click it and go to the "BLAST" page (<https://phytozome.jgi.doe.gov/pz/portal.html#!search?show=BLAST>).



4. At the left of the page, select and tick the target plant species, including *Marchantia polymorpha*, *Physcomitrella patens*, *Selaginella moellendorffii*, *Capsella rubella*, *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, and *Brachypodium arborescens*, which are used for the subsequent sequence blast.
5. At the right of the "BLAST" page, paste the *A. thaliana* PIN1 protein sequence as the query sequence. Concerning the Algorithm parameters, choose the "Proteome" as the "Target type" and use "BLASTP" as the designated "Program".

Search for genes, families and sequences

1. Select a Target 3 species selected ✕

Target set: Phytozome 12.1 Pre-release species

Target type: Ancestor nodes Species

Marchantia polymorpha v3.1

Viridiplantae

- Embryophyte**
 - Marchantia polymorpha v3.1 ✓
 - Physcomitrella patens v3.3 ✓
 - Sphagnum fallax v0.5
- Tracheophyte**
 - Selaginella moellendorffii v1.0 ✓
- Angiosperm**
 - Ananas comosus v3
 - Amborella trichopoda v1.0
 - Musa acuminata v1
 - Spirodela polyrhiza v2
 - Zostera marina v2.2
- Grass**

2. Build your query GO

Search type: Keyword BLAST

```
ILIMVWRKLRNPNSYSSLFGITWLSLISFKWNIEMPALIAKSISILSDAGLGMA
MFSLGLFMLNPRIACGNRRAAFAAAMRFVVGPAVMLVASYAVGLRGVLL
HVAIQAAALPQGIVPFVFAKEYNVHPDILSTAVIFGMLIALPITLLYILLGL
```

Algorithm parameters

Query name:

View results in browser

Notify by email (long or multifasta jobs)

Target type: Target type: Proteome

Program: BLASTP - protein query to protein db

Expect (E) threshold:

Comparison matrix: BLOSUM62

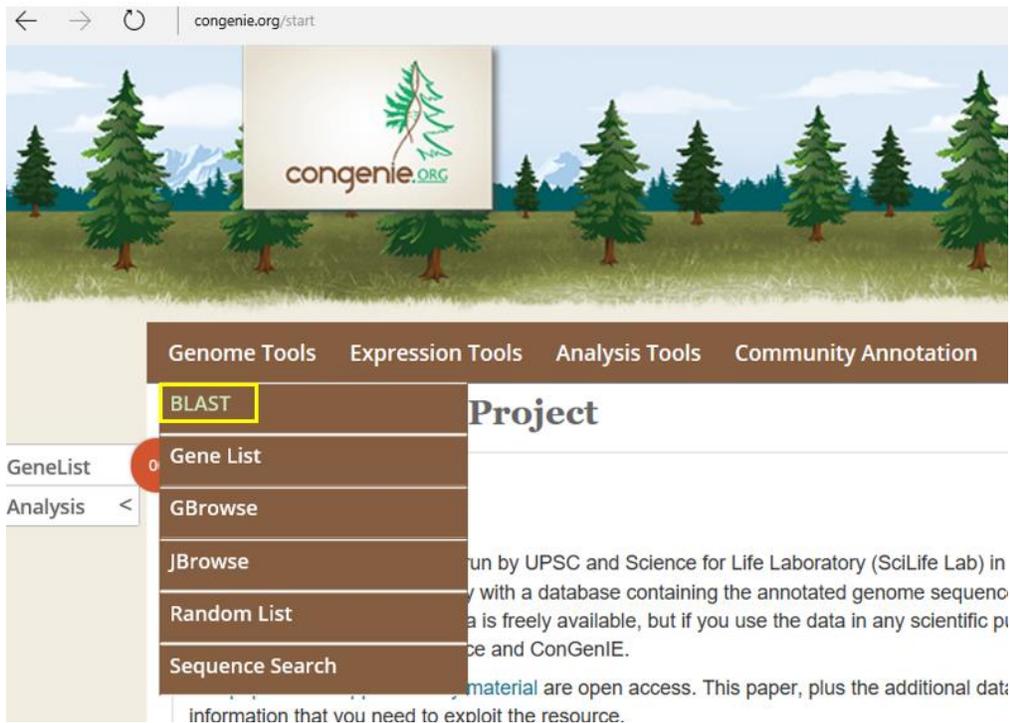
6. Click the “GO” button to start the query. In the BLAST results, a series of PIN members are extracted and listed according to the scores, which represent the sequence similarity between the query and hit PIN sequences among the selected Species.
7. Click the green button “G” to gain an overview of the selected gene .

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Views	Org	Defline	Score	E
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	G B	Mpo	Mapoly0089s0050.1 (1 of 2) K13947 - auxin efflux car...	634.0	0.0
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	G B	Smo	99301 (e_gw1.21.81.1) (1 of 5) K13947 - auxin efflux ...	617.8	0.0
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	G B	Smo	102666 (e_gw1.26.13.1) (1 of 5) K13947 - auxin efflu...	559.7	0.0
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	G B	Smo	119024 (e_gw1.59.169.1) (1 of 5) K13947 - auxin effl...	541.2	0.0
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	G B	Ppa	Pp3c10_24880V3.1 (Pp3c10_24880J4.1,Pp3c10_24...	538.5	0.0
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	G B	Ppa	Pp3c10_24880V3.3 (Pp1s32_43V6.1,Pp3c10_24880...	533.1	4.5E-180
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	G B	Ppa	Pp3c24_2970V3.2 (Pp3c24_2970P2.1,Pp1s18_186V...	377.5	8.5E-120
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	G B	Ppa	Pp3c24_2970V3.1 (Pp3c24_2970J4.1,Pp3c24_2970,...	377.5	8.5E-120

8. Find and click the menu “Sequence” in the page of gene view, where the PIN sequences from certain plant species can be visualized.

❖ **Search for PIN family members from the gymnosperm *Picea abies* and *Pinus taeda***

1. Open the homepage of Spruce Genome Project database (<http://congenie.org/start>).
2. Find the top menu "Genome Tools" and go to the main "BLAST" page (<https://congenie.org/blast>).



3. Enter the *Arabidopsis* PIN1 protein sequence as the query sequence.
4. Set up the BLAST program (choosing the "BLASTP") and select the blast database, which is denoted by "Select Blast DB" and can be found at the end of the webpage.

Load example:

BLAST program: ?

Query sequence:

```

MITAADFYHHVHTAMNPLYVAMILAYGSVKHMKIFTPDQCSGINRFVALFAVPLLSFHFIAANNPYAMNLRFLAADS LQKVIVLSLLFLWCKLSRNGSL
DHTITLFLSLSLTPNLTVMGIPLLKGHYGNFSGDLNMQIVLQCIIINVTLMFLFEYRGA LLISEQPDTAGSIVSIHVSDSDINSLDGRQPLETEAEI
KEDGKLVTVRRSNASRSDIYSRRSQGLSATPRPSNLTNAEISYLQSSRNPTPRGSSFNHTDFYSWASGGGRNSNFGPGEAVFGSKGPTPRPSNYEE
DGGPAKPTAAGTAAGARFHYQSGSGGGGGAHYAPNPGMFSPTNGGGGTAAGGNAPVVGGRQDGNRDLHMFWNISSASPVSDVFGGGGNHHA
DYSTATNDHQDKVKSIVPQGNNDNQYVEREFPFSGNKKDDSKVLATDGGNINSNKTQAKVMPPTSVNTRLILIMVNRKLRNPNYSVSLGITWLSL
ISFKWNIEMPALIAKSTISILSDAGLGMAMFSLGLFHALNPRIIACGNRRRAFAAAMRFVVGPAVMLVASVAVGLRGLVLLHVAIIQALPQGI VPFVFA
KEYNVHPDILSTAVIFGMLIALPITLLYYILLGL

```

Upload File: No file chosen ?

Select Blast DB:

- Gene Prediction (P. abies)
 - High Confidence Genes (protein sequence)
 - Medium Confidence Genes (protein sequence)
 - Low Confidence Genes (protein sequence)
 - All Gene Prediction (protein sequence)
 - Transposable element
- Gene Prediction (P. taeda)
 - Pinus taeda high quality genes
 - Pinus taeda low quality genes
 - Pinus taeda high quality partial genes
 - Pinus taeda low quality partial genes
- Putative Mitochondrial (P. abies)
 - Picea abies Putative Mitochondrial

+ Advance Search Parameters ?

5. Click the orange button “BLAST”. The extracted the PIN family members from *Picea abies* and *Pinus taeda* will be listed according to the scores based on the sequence similarity between PIN proteins from *Arabidopsis* and other species.
6. Tick the Query ID and click the button “FASTA” at the bottom of the page to visualize the sequences of blasted PIN proteins from *Picea abies* and *Pinus taeda*.

Overview of significant results for Query_1 Unknown_Query (query 1)

<input checked="" type="checkbox"/>	Query ID	Hit ID	Average bit score (top)	Average e-value (lowest)	Average identity (av. similarity)	Links
<input checked="" type="checkbox"/>	Query_1 Unknown_Query	MA_100472g0010	722.62 (722.62)	0.00e+0 (0.00e+0)	59.48 % (71.72 %)	GB
<input checked="" type="checkbox"/>	Query_1 Unknown_Query	PITA_000024726	708.75 (722.62)	0.00e+0 (0.00e+0)	58.29 % (68.71 %)	GB
<input checked="" type="checkbox"/>	Query_1 Unknown_Query	PITA_000003882	708.37 (722.62)	0.00e+0 (0.00e+0)	57.37 % (69.83 %)	GB
<input checked="" type="checkbox"/>	Query_1 Unknown_Query	MA_69724g0010	639.42 (722.62)	0.00e+0 (0.00e+0)	56.01 % (66.52 %)	GB
<input checked="" type="checkbox"/>	Query_1 Unknown_Query	PITA_000013100	327.41 (722.62)	2.13e-65 (0.00e+0)	61.38 % (72.52 %)	GB
<input checked="" type="checkbox"/>	Query_1 Unknown_Query	PITA_000046447	220.13 (722.62)	2.16e-18 (0.00e+0)	46.57 % (61.13 %)	GB
<input checked="" type="checkbox"/>	Query_1 Unknown_Query	MA_61553g0010	240.74 (722.62)	7.94e-73 (0.00e+0)	49.68 % (60.71 %)	GB
<input checked="" type="checkbox"/>	Query_1 Unknown_Query	PITA_000012975	213.19 (722.62)	1.04e-53 (0.00e+0)	41.97 % (57.07 %)	GB

Download or Export hits of selected results

If the reference database has been indexed then you can download the hits or export to Congenie Galaxy.

❖ **Search for PIN family members from *Amborella trichopoda*.**

1. Go to the Amborella database, which is included in the EnsemblPlants database (https://plants.ensembl.org/Amborella_trichopoda/Info/Annotation/).
2. Find the top menu "BLAST" and click it to link to the main EnsemblPlants "BLAST" page (https://plants.ensembl.org/Amborella_trichopoda/Tools/Blast).

Amborella trichopoda Assembly and Gene Annotation

About *Amborella trichopoda*

Amborella trichopoda is a small, tropical shrub endemic to New Caledonia. It is the only species in the genus *Amborella*, which is the only member of the family Amborellaceae. As the only living species on the sister lineage to all other flowering plants, it is an important reference for studying plant evolution. Individual *Amborella trichopoda* are usually sprawling understory shrubs, although occasionally they grow up to eight metres high. They have evergreen leaves and small, white to yellow flowers. The species is dioecious; female plants producing carpals and males producing stamens. Individual plants can change sex between flowerings. Unlike nearly all other flowering plants, they do not possess vessel elements for water conduction.

Amborella's genome is a relatively compact 870Mb, arranged into 13 chromosome pairs.

3. Paste the *Arabidopsis* PIN1 protein sequence into the blank box at the right of "Sequence data".
4. Choose the "BLASTP" as the search tool.

BLAST search

New job

Sequence data:

```
>
MITAADFYHVTAMVPLYVAMILAYGSVMWMI FTPDQCSGINRFVALFAVPLLSFHFIA
AMNPHYAMNLRFLAADS LQKVI VLSLLFLWCKLSRNGSLDWTITLFSLSLTPNLLVMGIPL
LKGMYGNFSGDLMVQIVVLQCI IWYTLMLFLFEYRGAKLLI SEQFFDTAGSIVSIHVDS
IMSLDGRQPLETEAEIKEDGKLVTVRRSNASRSDIYSRRSQGLSATPRPSNLTNAEIS
LQSSRNPTPRGSSFNHTDFYSMMASGGGRNSNFGPGEAVFGSKGPTPRPSNYEEDGGPAK
PTAAGTAAAGAGRFHYQSGSGGGGGAHYAPNPGMFSFNTGGGGTAAKGNAPVVGKQK
DGNGRDLHMFVWSSASFSVSDVFGGGGNHHDYSTATNDHQKDVKI SVFQGNNDNQYV
EREFFSFGNKDDSKVLATDGGNNISNKTQAKVMPPTSMVTRLILIMVWRKLRNPNYS
SSLFGITWLSLISFKWNIEMPALIAKISISILSDAGLGMAMFSLGLFMALNPRIIACGNRRA
AFAAAMRFVVGPAVMLVASVAVGLRGLLHVAI IQAALPQGIVPFVFAKEYNVHPDILST
AVIFGMLIALPITLLYYILLGL
```

[Add more sequences](#) (29 more sequences allowed)

Protein
 DNA

Search against:

 Amborella_trichopoda X
[Add/remove species](#)

DNA database Genomic sequence
 Protein database Proteins

Search tool: BLASTP

Search Sensitivity: Normal

5. Click the green button “Run” to start the sequence alignment.
6. Choose the “View results” to jump to the page of BLAST result, where the sequences of PIN family members from *Amborella trichopoda* can be found.

BLAST search

New job

Recent jobs

Refresh

Show/hide columns (1 hidden)

Analysis	Jobs
BLASTP	 BLASTP against Amborella trichopoda AMTR1.0 (Proteins) Done: 32 hits found View results