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Expression Atlas: exploring plant gene expression results

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This practical will introduce you to the data content and functionality of Expression Atlas (www.ebi.ac.uk/gxa/home) at EMBL-EBI.

More information can be found on:
3. Expression Atlas: Quick tour:
   https://www.ebi.ac.uk/training/online/course/expression-atlas-quick-tour-1
4. Expression Atlas help page: https://www.ebi.ac.uk/gxa/help/index.html
5. Expression Atlas FAQ section: https://www.ebi.ac.uk/gxa/FAQ.html

Exercise 1

Baseline Expression – Gene expression patterns of members of the PIN gene family in maize

Scenario

The PIN-FORMED (PIN) proteins are secondary transporters acting in the efflux of the plant signal molecule auxin from cells. The first PIN family members identified and associated with auxin transport were described in the model plant *Arabidopsis thaliana*. Arabidopsis has eight annotated *PIN* genes, *PIN1-PIN8*. You are working on a research project to characterize *PIN* family members in *Zea mays*. As part of this project you are interested on exploring expression pattern of each member of the *PIN* gene family in maize.

One of the first *PIN* genes characterized in maize was *ZmPIN1a* (Carraro et al, 2006). Let's start exploring PIN gene family in maize by searching for *ZmPIN1a* in Expression Atlas.

Tasks – Gene search

Use the **Gene / Gene properties search box** on Expression Atlas home page to search for *ZmPIN1a* gene. Start typing the first characters of *ZmPIN1a* gene and you will see a list of suggested genes including it. As you want to explore the results in maize you can be more specific in your search selecting *Zea mays* as organism.

First of all, you will see that *ZmPIN1a* gene has been changed into *Zm00001d044812*. Let's go to the **PIN-formed protein1 information** tab to see why. In this tab you can see that *ZmPIN1a* gene is a synonym of the Ensembl gene *Zm00001d044812*. Expression Atlas imports plant gene annotations from Ensembl Plants at EMBL-EBI (https://plants.ensembl.org/index.html).

Let's explore now the **Baseline expression** tab. The Baseline Expression results will display all tissues (columns) studied in different experiments (rows) in which *Zm00001d044812* gene is expressed above minimum expression level (0.5 TPM).
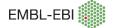
Tasks – Baseline expression results

1. Is *Zm00001d044812* gene expressed in pollen? Do the experiments agree on this?

2. What tissue/s show higher expression of *Zm00001d044812* gene?
   Hint: You can sort the heatmap results by '**Expression rank**' using one of the buttons above the heatmap.

By default we display expression data for different tissues (organism part) but you can use the filters in the left to explore *Zm00001d044812* expression in other conditions such as cell type and developmental stage. If you click on Developmental stage you will see an experiment from Stelpflug *et al.* covering lots of tissues and developmental stages. Let's click on the title "Spelpflug et al – seed" to open the experiment page.

Tasks – Baseline experiment page

6. Let's have a look at the gene expression data from the experiment "*Transcription profiling by high throughput sequencing in different developmental stages of Zea mays subsp. mays tissues*"
   a. What is the expression level in seed at 2 days after pollination?
   b. What is the expression pattern of *Zm00001d044812* gene across seed development?

7. Let's explore variation of *Zm00001d044812* gene expression among biological replicates. How many biological replicates were tested for that particular condition, seed at 2 days after pollination?
   a. What is the maximum expression value among them?
      Hint: Click on **Switch to boxplot and transcript view**

8. How many transcripts does *Zm00001d044812* gene have?

9. Now, let's find more information about *Zm00001d044812* gene. In particular we want to see *Zm00001d044812* gene in the context of the genomic location of the gene. First, switch to the heatmap view. Now, click on 'Select genome browser to view tracks' button above the heatmap, select '**Ensembl genome browser**' and click on a cell of the heatmap (e.g. seed, 2 days after pollination).
   a. On which chromosome is *Zm00001d044812* gene located?
   b. What is the length of *Zm00001d044812* gene?

c. How many exons does *Zm00001d044812* gene have?
d. How many orthologues to *Zm00001d044812* gene have been found?
e. What about paralogues? Can you get all the gene identifiers for the paralogues to *Zm00001d044812* gene?
f. Plant specific PIN family of efflux carriers comprises integral membrane proteins. Can you find how many transmembrane helices were predicted for Zm00001d044812 protein?

10. Now, we can explore gene expression of paralogues of *Zm00001d044812* gene in Expression Atlas. We need to start typing the Ensembl identifiers in the **Genes** search box that appears in the left of the experiment page in Expression Atlas.
Hint: Copy and paste the Ensembl identifiers (one by one) for the each of the members of the PIN gene family in the Genes search box, select the gene from the suggested list and click on Apply.

Ensembl identifier	Gene name
Zm00001d018024	PIN-formed protein2
Zm00001d052269	PIN-formed protein3
Zm00001d052442	PIN-formed protein4
Zm00001d046893	Auxin efflux carrier component 2
Zm00001d045219	PIN-formed protein12
Zm00001d044083	PIN-formed protein10
Zm00001d043660	PIN-formed protein8
Zm00001d043179	PIN-formed protein9
Zm00001d031594	PIN-formed protein7
Zm00001d042345	PIN-formed protein5
Zm00001d006082	PIN-formed protein11

a. Is there any "*PIN-formed protein*" gene that is not expressed in seed in any of the developmental stages studied?

11. What is the expression profile of all the maize PIN family genes across leaf development?
Hint: Use the **Select** button under Experimental variables in the left to select leaf as organism part instead of seed.

12. What about displaying gene expression pattern of PIN family genes in all tissues and in all developmental stages?
Hint: Use the **Select** button under Experimental variables in the left to choose all.

In this exercise we have used Expression Atlas to find out in which conditions (tissues and developmental stages in that example) is a particular gene or set of genes expressed. To answer this kind of questions we use the Gene /Gene properties search box on the home page. We can also use Expression Atlas in a baseline context to answer questions such as what genes are expressed in a particular condition? For example, what genes are expressed in **rice endosperm**? To answer this kind of questions we use the Biological condition search box on the home page.

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