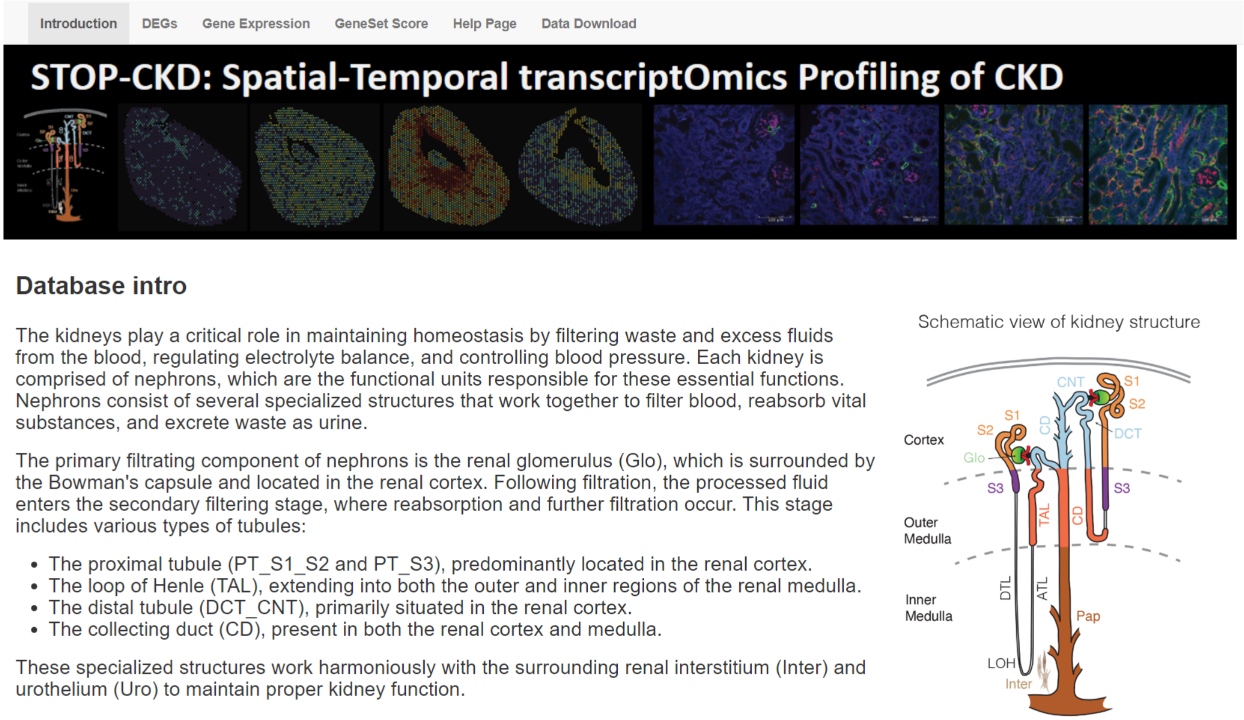
How to Use the STOP-CKD Database

The STOP-CKD (Spatial-Temporal transcriptOmics Profiling of CKD) database is designed to help researchers and clinicians explore gene expression profiles in the context of chronic kidney disease. Here’s a practical guide on how to navigate and utilize the database effectively:

Accessing the Database

1. **Navigate the Interface**: Use the tabs at the top of the page to switch between different sections of the database: Introduction, DEGs (Differentially Expressed Genes), Gene Expression, GeneSet Score, Help Page, and Data Download.



STOP-CKD: Spatial-Temporal transcriptOmics Profiling of CKD

Database intro

The kidneys play a critical role in maintaining homeostasis by filtering waste and excess fluids from the blood, regulating electrolyte balance, and controlling blood pressure. Each kidney is comprised of nephrons, which are the functional units responsible for these essential functions. Nephrons consist of several specialized structures that work together to filter blood, reabsorb vital substances, and excrete waste as urine.

The primary filtrating component of nephrons is the renal glomerulus (Glo), which is surrounded by the Bowman's capsule and located in the renal cortex. Following filtration, the processed fluid enters the secondary filtering stage, where reabsorption and further filtration occur. This stage includes various types of tubules:

- The proximal tubule (PT_S1_S2 and PT_S3), predominantly located in the renal cortex.
- The loop of Henle (TAL), extending into both the outer and inner regions of the renal medulla.
- The distal tubule (DCT_CNT), primarily situated in the renal cortex.
- The collecting duct (CD), present in both the renal cortex and medulla.

These specialized structures work harmoniously with the surrounding renal interstitium (Inter) and urothelium (Uro) to maintain proper kidney function.

How to Use Differentially Expressed Genes (DEGs) Tab

The DEGs (Differentially Expressed Genes) tab in the STOP-CKD database allows users to explore genes that exhibit significant changes in expression levels across different kidney regions and time points. Here's a detailed guide on how to use this feature:

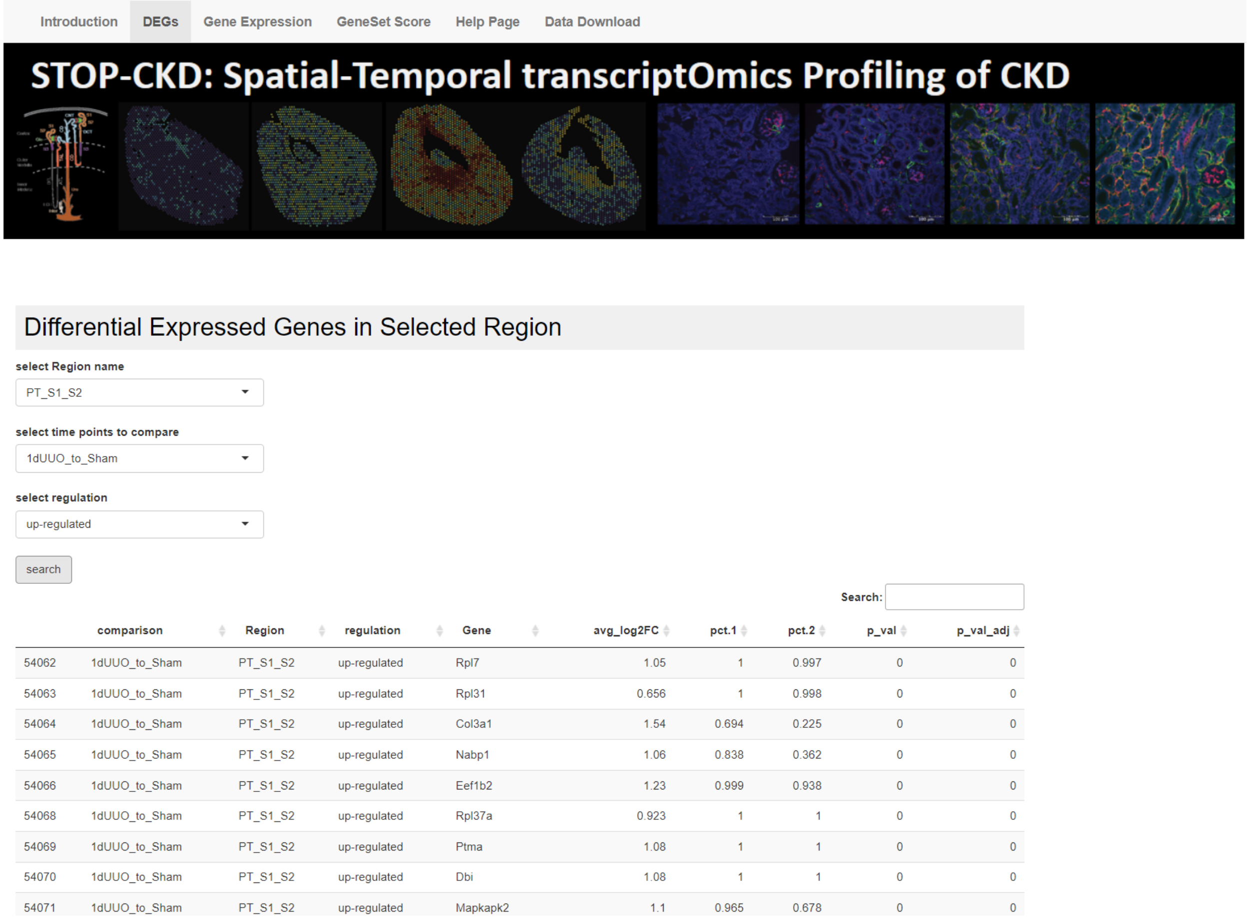
Accessing Differentially Expressed Genes

1. **Select the DEGs Tab**: Click on the "DEGs" tab on the top navigation bar to open the DEGs analysis interface.

Using the DEGs Interface

1. **Select Region Name**:
    - Use the dropdown menu under "select Region name" to choose the specific kidney region you are interested in. For example, you might select "PT_S1_S2" to focus on the proximal tubule segments S1 and S2.
2. **Select Time Points to Compare**:
    - Use the second dropdown menu to choose the time points you wish to compare. For instance, selecting "1dUUO_to_Sham" compares gene expression between the 1-day unilateral ureteral obstruction (UUO) model and the sham-operated control.
3. **Select Regulation**:
    - Use the third dropdown menu to specify the type of regulation you are interested in. You can choose "up-regulated" to see genes that are expressed at higher levels or "down-regulated" for those expressed at lower levels in the selected condition.
4. **Search and View Results**:
    - Click the "search" button to generate a list of differentially expressed genes based on your selected criteria.
    - The results will be displayed in a table, showing various columns such as:
        - **Comparison**: The comparison made (e.g., 1dUUO_to_Sham).
        - **Region**: The specific kidney region selected.
        - **Regulation**: Whether the gene is up-regulated or down-regulated.
        - **Gene**: The name of the gene.
        - **avg_log2FC**: The average log2 fold change in expression.
        - **pct.1**: The percentage of cells expressing the gene in the first condition.
        - **pct.2**: The percentage of cells expressing the gene in the second condition.
        - **P_val**: The p-value indicating the statistical significance of the expression change.
        - **P_val_adj**: The adjusted p-value for multiple comparisons.

By following these steps, you can efficiently navigate the DEGs tab in the STOP-CKD database to identify and analyze genes with significant expression changes in different kidney regions and disease conditions. This information can be crucial for understanding the molecular mechanisms of CKD and identifying potential targets for therapeutic intervention.



Differential Expressed Genes in Selected Region

select Region name: PT_S1_S2

select time points to compare: 1dUUO_to_Sham

select regulation: up-regulated

	comparison	Region	regulation	Gene	avg_log2FC	pct.1	pct.2	p_val	p_val_adj
54062	1dUUO_to_Sham	PT_S1_S2	up-regulated	Rpl7	1.05	1	0.997	0	0
54063	1dUUO_to_Sham	PT_S1_S2	up-regulated	Rpl31	0.656	1	0.998	0	0
54064	1dUUO_to_Sham	PT_S1_S2	up-regulated	Col3a1	1.54	0.694	0.225	0	0
54065	1dUUO_to_Sham	PT_S1_S2	up-regulated	Nabp1	1.06	0.838	0.362	0	0
54066	1dUUO_to_Sham	PT_S1_S2	up-regulated	Eef1b2	1.23	0.999	0.938	0	0
54068	1dUUO_to_Sham	PT_S1_S2	up-regulated	Rpl37a	0.923	1	1	0	0
54069	1dUUO_to_Sham	PT_S1_S2	up-regulated	Ptma	1.08	1	1	0	0
54070	1dUUO_to_Sham	PT_S1_S2	up-regulated	Dbi	1.08	1	1	0	0
54071	1dUUO_to_Sham	PT_S1_S2	up-regulated	Mapkapk2	1.1	0.965	0.678	0	0

How to Use Gene Expression Tab

The Gene Expression tab in the STOP-CKD database provides detailed information on gene expression profiles across different regions and conditions. Here’s a practical guide on how to use this feature effectively:

Accessing Gene Expression Data

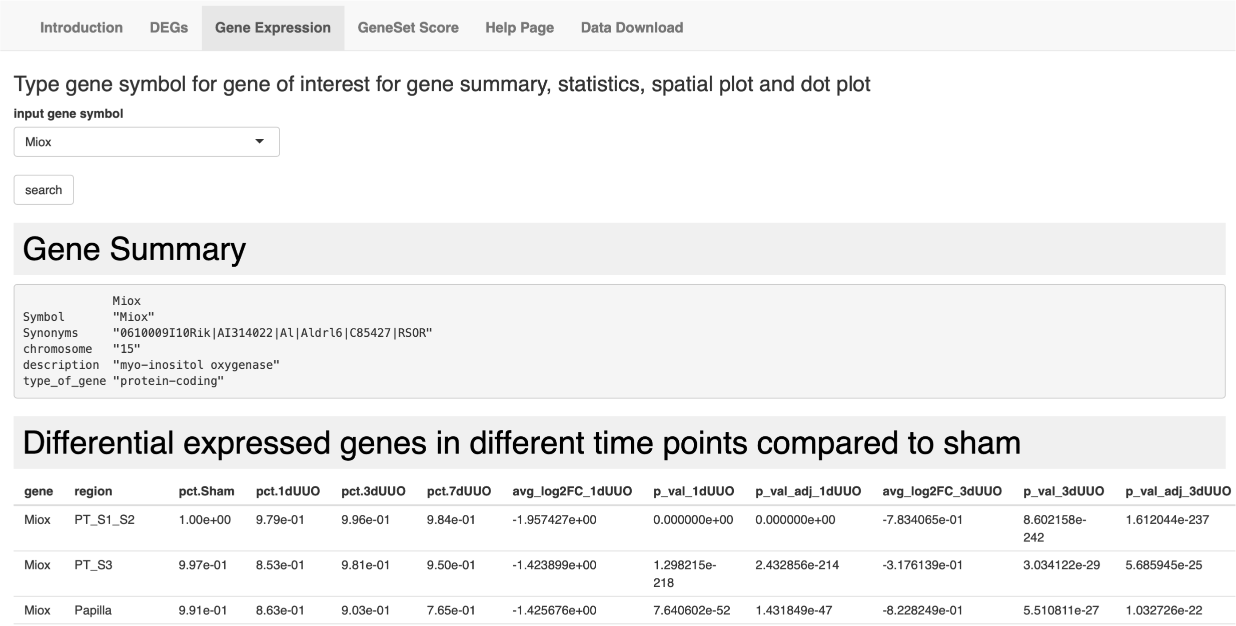
1. **Select the Gene Expression Tab**: Click on the "Gene Expression" tab on the top navigation bar to open the gene expression analysis interface.

Searching for a Specific Gene

1. **Input Gene Symbol**:
    - Type the gene symbol of interest in the input box (e.g., "Miox").
    - Click the "search" button to retrieve the gene expression data.
2. **Gene Summary**:
    - After searching, a summary of the gene will be displayed, including its symbol, synonyms, chromosome location, description, and type.

Exploring Differentially Expressed Genes

1. **Differentially Expressed Genes Table**:
    - This table shows the expression levels of the selected gene across different regions and time points compared to sham controls.
    - Key columns include:
        - **gene**: The gene symbol.
        - **region**: The kidney region.
        - **pct.Sham**: The percentage of cells expressing the gene in the sham condition.
        - **pct.1dUUO**: The percentage of cells expressing the gene in the 1-day UUO condition.
        - **avg_log2FC**: The average log2 fold change in expression.
        - **p_val** and **p_val_adj**: The p-value and adjusted p-value indicating the statistical significance of the expression change.



Type gene symbol for gene of interest for gene summary, statistics, spatial plot and dot plot

input gene symbol: Miox

Gene Summary

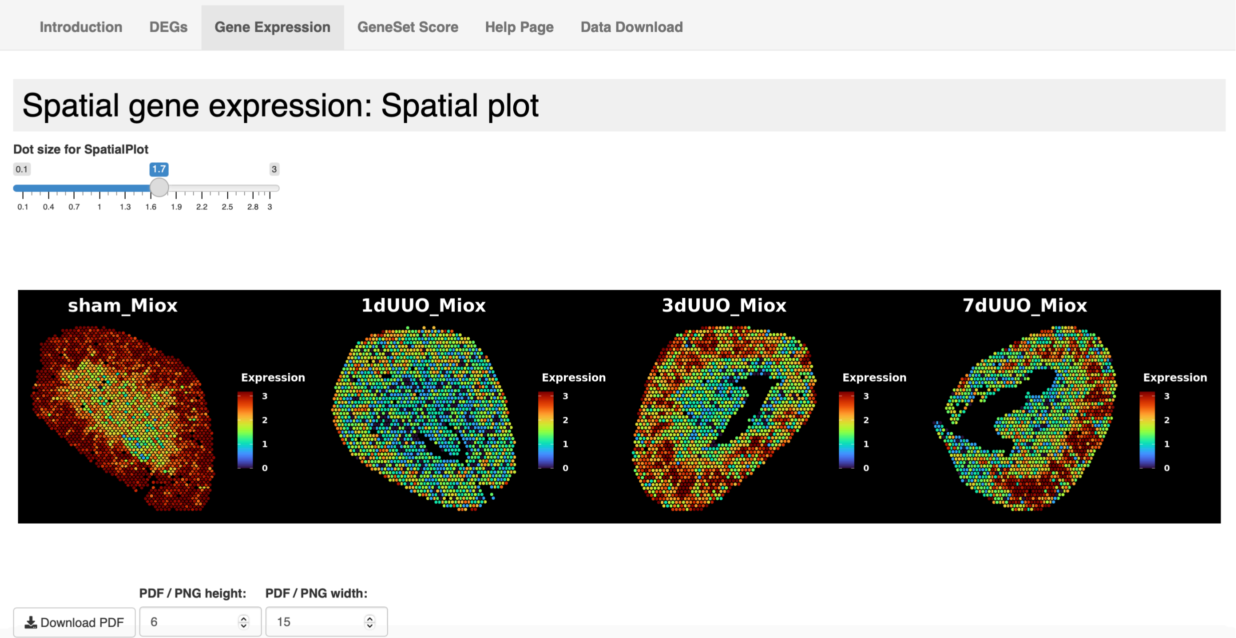
```
             Miox
Symbol       "Miox"
Synonyms     "0610009I10Rik|AI314022|Al|Aldrl6|C85427|RSOR"
chromosome   "15"
description  "myo-inositol oxygenase"
type_of_gene "protein-coding"
```

Differential expressed genes in different time points compared to sham

gene	region	pct.Sham	pct.1dUUO	pct.3dUUO	pct.7dUUO	avg_log2FC_1dUUO	p_val_1dUUO	p_val_adj_1dUUO	avg_log2FC_3dUUO	p_val_3dUUO	p_val_adj_3dUUO
Miox	PT_S1_S2	1.00e+00	9.79e-01	9.96e-01	9.84e-01	-1.957427e+00	0.000000e+00	0.000000e+00	-7.834065e-01	8.602158e-242	1.612044e-237
Miox	PT_S3	9.97e-01	8.53e-01	9.81e-01	9.50e-01	-1.423899e+00	1.298215e-218	2.432856e-214	-3.176139e-01	3.034122e-29	5.685945e-25
Miox	Papilla	9.91e-01	8.63e-01	9.03e-01	7.65e-01	-1.425676e+00	7.640602e-52	1.431849e-47	-8.228249e-01	5.510811e-27	1.032726e-22

Visualizing Spatial Gene Expression

1. **Spatial Plot**:
   - This section provides a visual representation of the gene expression in spatial context.
   - Adjust the dot size using the slider to change the visualization resolution.
   - Download the spatial plot as a PDF or PNG file by setting the desired dimensions and clicking the download buttons.



Spatial gene expression: Spatial plot

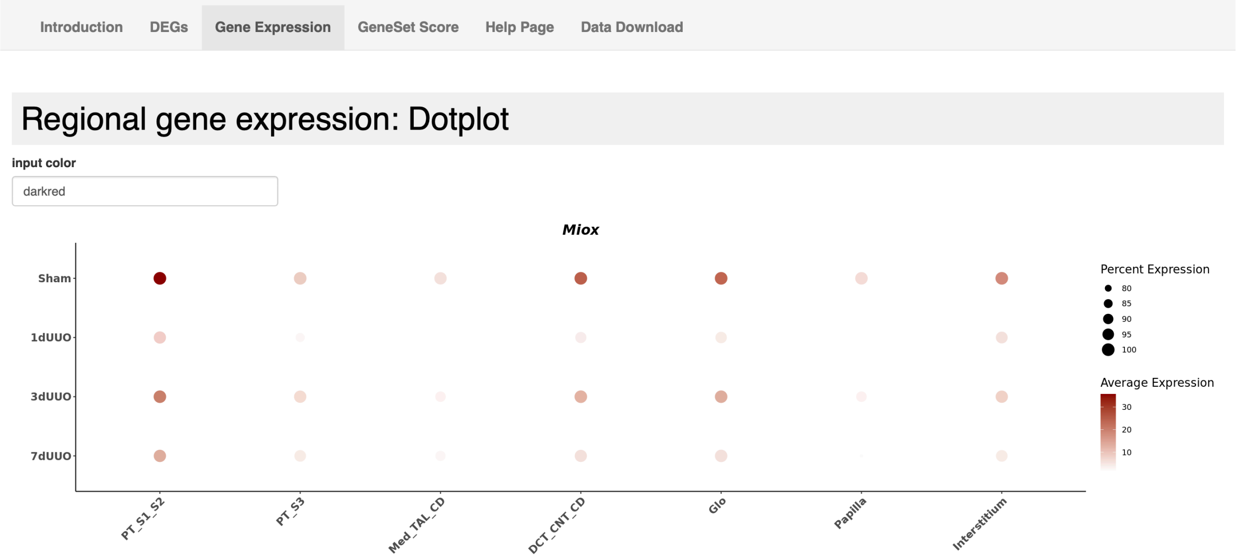
Dot size for SpatialPlot: 1.7

PDF / PNG height: 6

PDF / PNG width: 15

Creating Dotplots for Regional Gene Expression

1. **Dotplot**:
    - This section allows you to visualize gene expression across different regions and conditions in a dotplot format.
    - Select the color for the dots using the input color box.
    - The dot size indicates the percentage of cells expressing the gene, while the color intensity represents the average expression level.

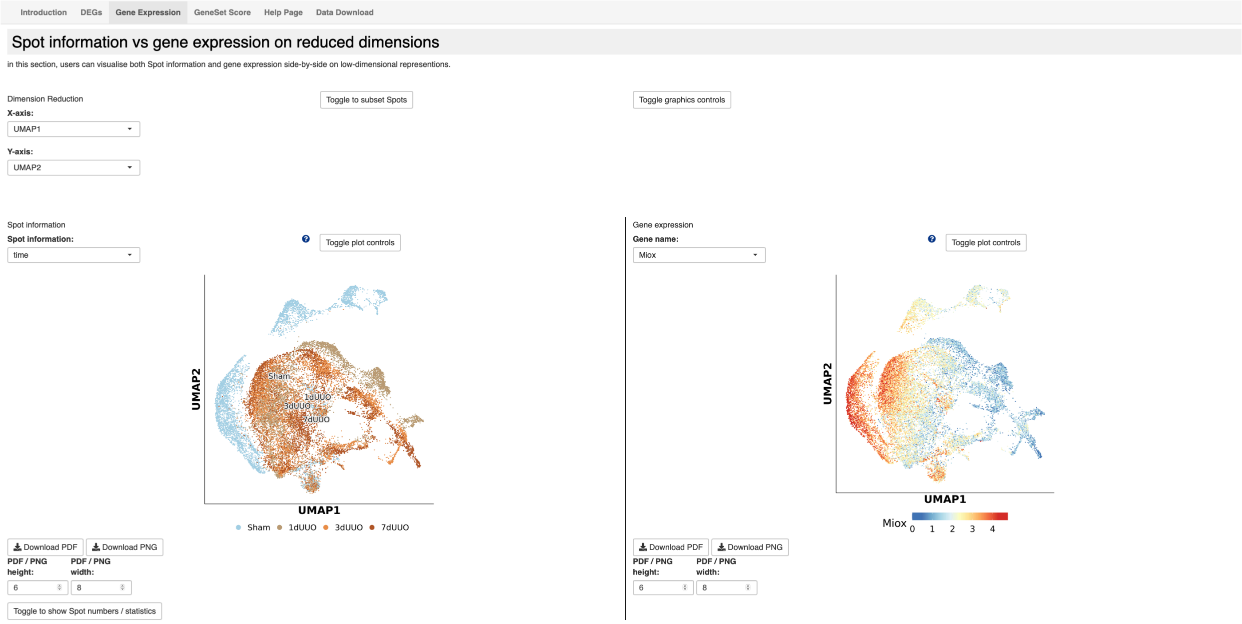


Regional gene expression: Dotplot

input color: darkred

Analyzing Gene Expression on Reduced Dimensions

1. **Dimension Reduction Plot**:
    - Use this section to visualize gene expression on reduced dimensions (e.g., UMAP).
    - Select the axes for dimension reduction (e.g., UMAP1 and UMAP2).
    - Toggle between different spot information and gene expression views.
    - Download the plots as PDF or PNG files.



Spot information vs gene expression on reduced dimensions

in this section, users can visualise both Spot information and gene expression side-by-side on low-dimensional representions.

Dimension Reduction

X-axis: UMAP1

Y-axis: UMAP2

Spot information

Spot information: time

Gene expression

Gene name: Miox

PDF / PNG height: 6

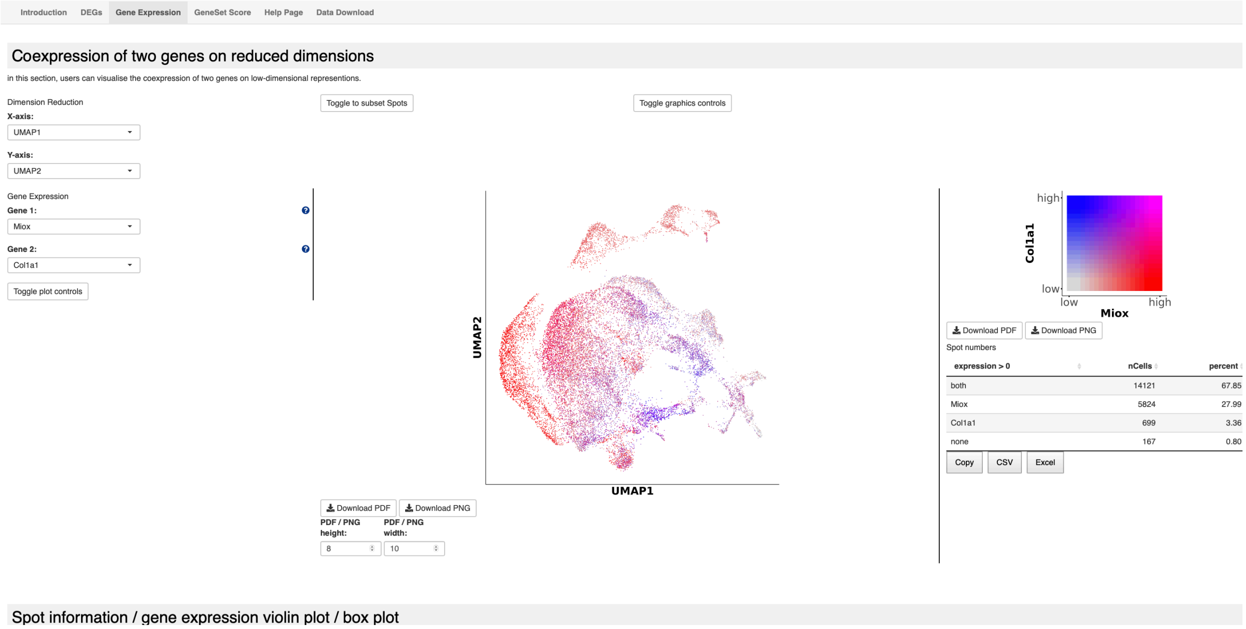
PDF / PNG width: 8

PDF / PNG height: 6

PDF / PNG width: 8

Co-expression of Two Genes

1. **Co-expression Plot**:
   - This section allows you to analyze the co-expression of two genes on reduced dimensions.
   - Select the genes to be analyzed (e.g., "Miox" and "Col1a1").
   - The plot shows the spatial distribution of cells expressing both genes.
   - Download the co-expression plot as a PDF or PNG file.



Coexpression of two genes on reduced dimensions

in this section, users can visualise the coexpression of two genes on low-dimensional representions.

Dimension Reduction

X-axis: UMAP1

Y-axis: UMAP2

Gene Expression

Gene 1: Miox

Gene 2: Col1a1

PDF / PNG height: 8

PDF / PNG width: 10

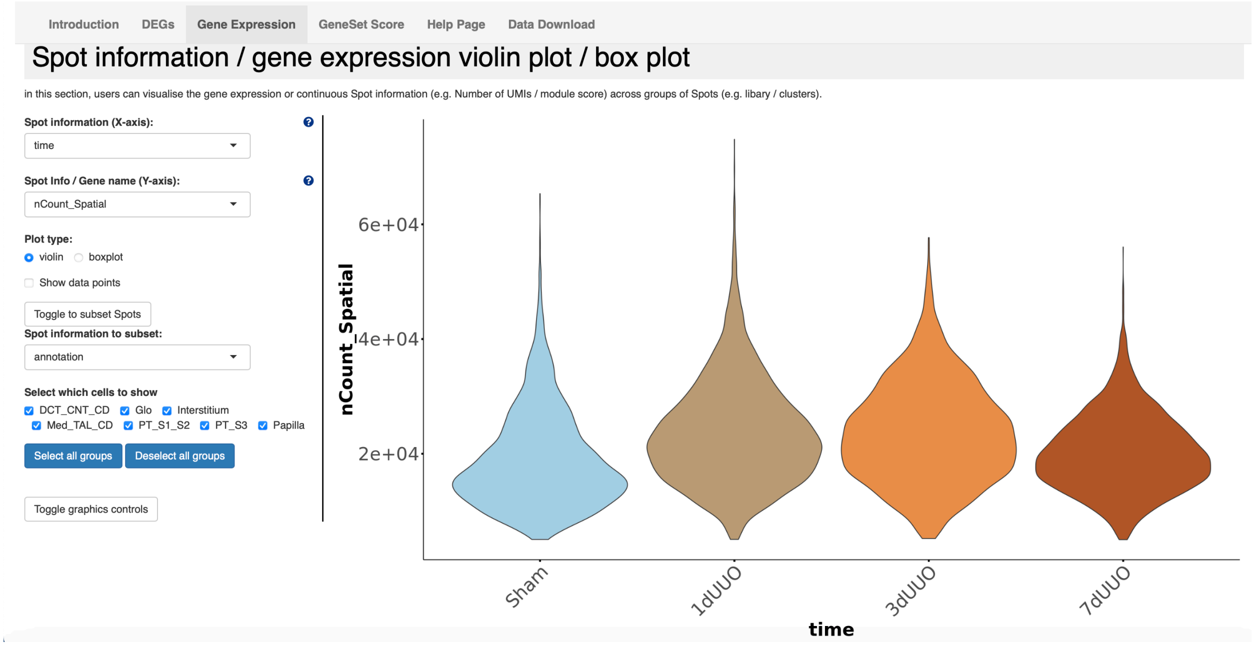
Spot numbers

expression > 0	nCells	percent
both	14121	67.85
Miox	5824	27.99
Col1a1	699	3.36
none	167	0.80

Spot information / gene expression violin plot / box plot

Visualizing Gene Expression with Violin and Box Plots

1. **Violin/Box Plot**:
   - This section provides a visual representation of gene expression distribution using violin or box plots.
   - Select the X-axis and Y-axis parameters, and choose the plot type (violin or boxplot).
   - Toggle between showing data points and adjusting graphics controls.
   - Download the plots as PDF or PNG files.



Spot information / gene expression violin plot / box plot

in this section, users can visualise the gene expression or continuous Spot information (e.g. Number of UMIs / module score) across groups of Spots (e.g. libary / clusters).

Spot information (X-axis): time

Spot Info / Gene name (Y-axis): nCount_Spatial

Plot type: ● violin ○ boxplot

☐ Show data points

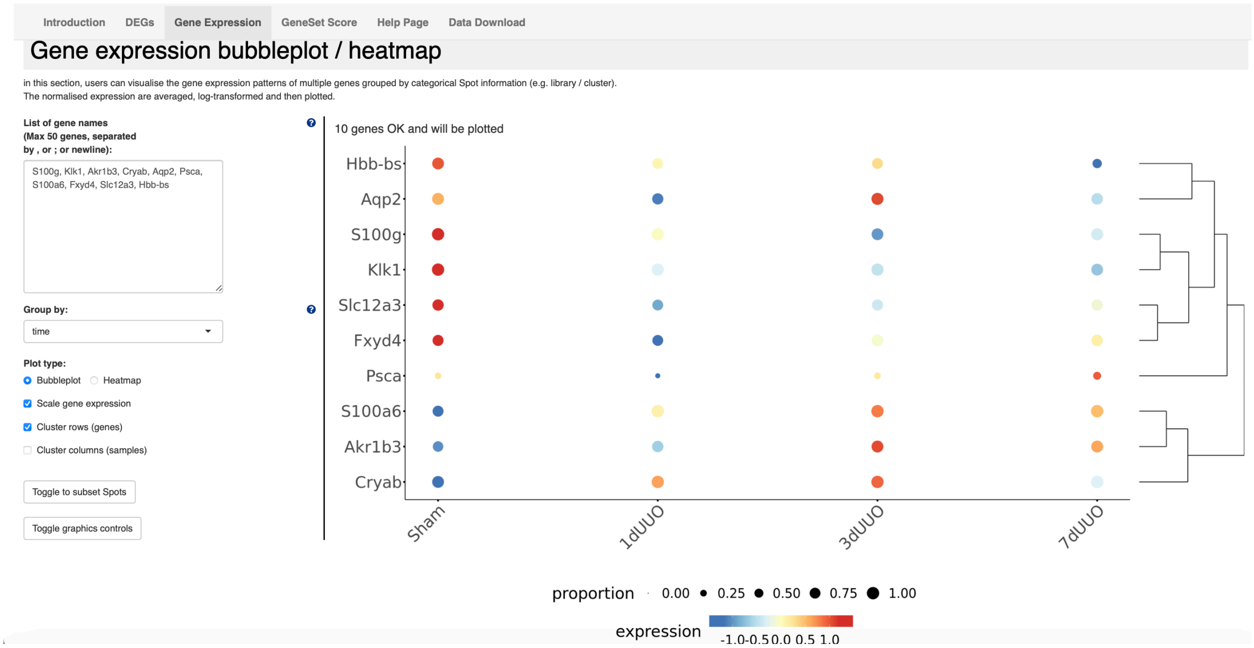
Spot information to subset: annotation

Select which cells to show

☑ DCT_CNT_CD ☑ Glo ☑ Interstitium ☑ Med_TAL_CD ☑ PT_S1_S2 ☑ PT_S3 ☑ Papilla

Creating Gene Expression Bubbleplots/Heatmaps

1. **Bubbleplot/Heatmap**:
   - This section allows you to visualize gene expression patterns of multiple genes.
   - Enter a list of gene names and select the grouping parameter (e.g., time).
   - Choose the plot type (bubbleplot or heatmap).
   - Cluster rows (genes) and columns (samples) for better visualization.
   - Download the plots as PDF or PNG files.



Gene expression bubbleplot / heatmap

in this section, users can visualise the gene expression patterns of multiple genes grouped by categorical Spot information (e.g. library / cluster).
The normalised expression are averaged, log-transformed and then plotted.

List of gene names (Max 50 genes, separated by , or ; or newline): S100g, Klk1, Akr1b3, Cryab, Aqp2, Psca, S100a6, Fxyd4, Slc12a3, Hbb-bs

Group by: time

Plot type: ● Bubbleplot ○ Heatmap

☑ Scale gene expression

☑ Cluster rows (genes)

☐ Cluster columns (samples)

10 genes OK and will be plotted

The STOP-CKD database includes a powerful subset function that allows users to focus their analysis on specific groups of cells within the kidney. This function can be applied to various visualization tools, including UMAP plots, violin plots, dot plots, and heatmaps. Here’s a detailed guide on how to use this feature:

Accessing the Subset Function

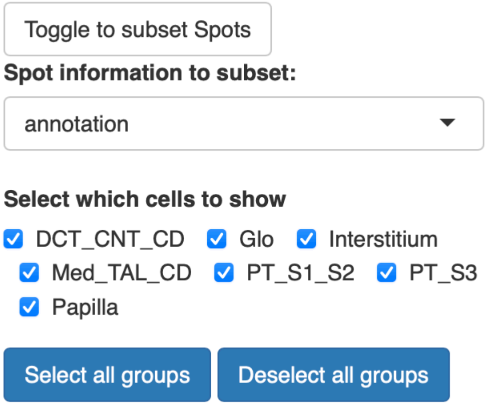
1. **Subset Function Panel**:
   - Located within the Gene Expression tab, the subset function panel allows you to filter which cells are included in your analysis.

Selecting Cell Subsets

1. **Spot Information to Subset**:
   - Use the dropdown menu to select the type of spot information you want to subset by (e.g., annotation).
2. **Select Which Cells to Show**:
   - Check or uncheck the boxes next to the spot groups you want to include in your analysis.
3. **Select/Deselect All Groups**:
   - Quickly include or exclude all cell groups by clicking "Select all groups" or "Deselect all groups."
4. **Toggle Graphics Controls**:
   - Click this button to access additional graphics settings for customizing your plots.

Applying Subset to Visualizations

Once you have selected the desired cell subsets, you can apply this filtering to various visualizations in the Gene Expression tab: UMAP Plot, Violin/Box Plot, Heatmap/Bubbleplot etc.



Spot information to subset: annotation

Select which cells to show

☑ DCT_CNT_CD ☑ Glo ☑ Interstitium
☑ Med_TAL_CD ☑ PT_S1_S2 ☑ PT_S3
☑ Papilla

How to Use GeneSet Score Tab

The GeneSet Score tab in the STOP-CKD database allows users to visualize gene set scores across different kidney regions and conditions. This feature helps in understanding the collective behavior of groups of genes involved in specific biological processes or pathways. Here’s a detailed guide on how to use this feature effectively:

Accessing GeneSet Scores

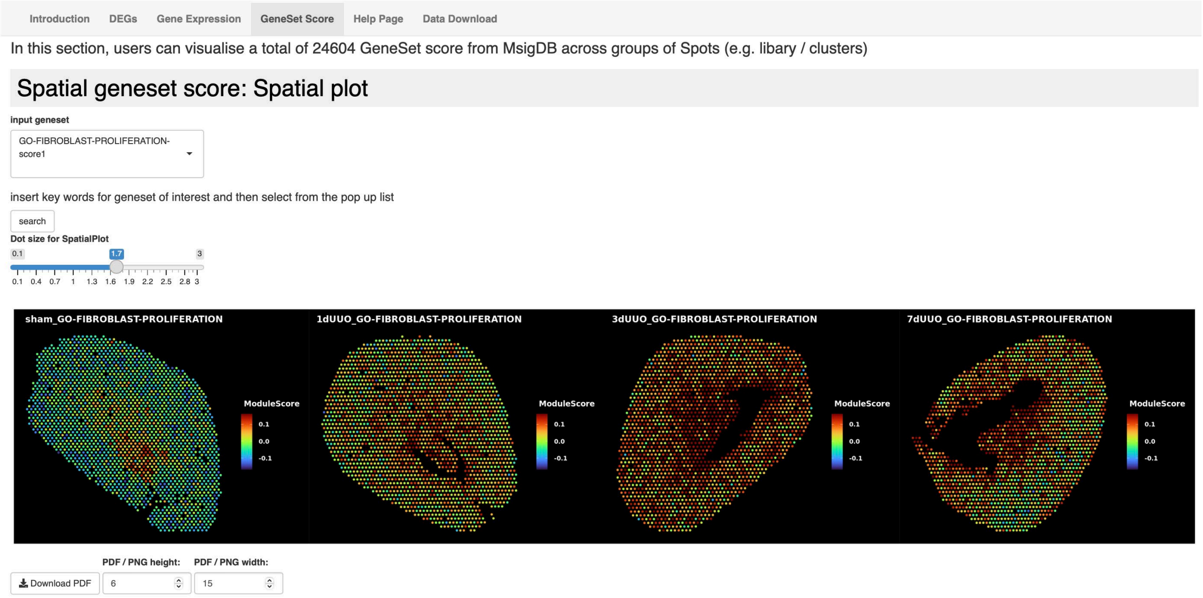
1. **Select the GeneSet Score Tab**: Click on the "GeneSet Score" tab on the top navigation bar to open the gene set score analysis interface.

Searching for a Gene Set

1. **Input Gene Set**:
   - Use the dropdown menu under "input geneset" to select a gene set of interest (e.g., "GO-FIBROBLAST-PROLIFERATION-score1").
   - Alternatively, you can type keywords related to your gene set of interest in the search box and select from the suggested options.
   - Click the "search" button to retrieve the gene set score data.

Visualizing GeneSet Scores

1. **Spatial Plot**:
   - The spatial plot provides a visual representation of the gene set scores across different conditions and time points.
   - Adjust the dot size for the spatial plot using the slider to change the visualization resolution.
   - The example spatial plot displays the gene set score for "GO-FIBROBLAST-PROLIFERATION" across sham, 1dUUO, 3dUUO, and 7dUUO conditions.
   - Download the spatial plot as a PDF or PNG file by setting the desired dimensions and clicking the download buttons.



In this section, users can visualise a total of 24604 GeneSet score from MsigDB across groups of Spots (e.g. libary / clusters)

Spatial geneset score: Spatial plot

input geneset: GO-FIBROBLAST-PROLIFERATION-score1

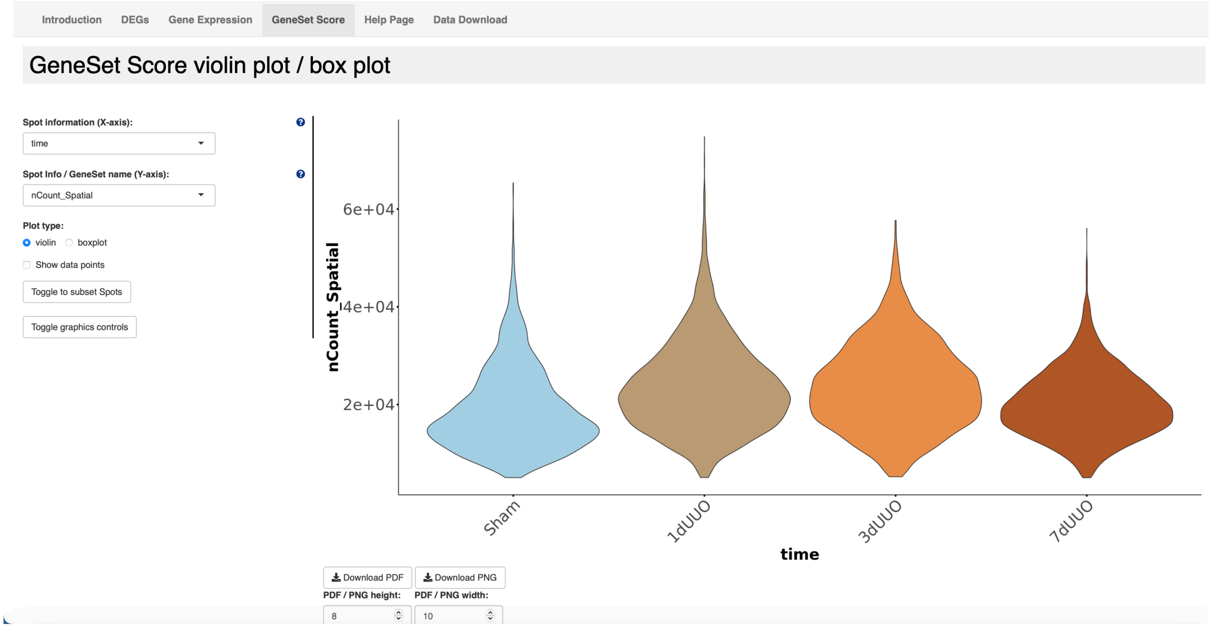
insert key words for geneset of interest and then select from the pop up list

Dot size for SpatialPlot: 1.7

PDF / PNG height: 6

PDF / PNG width: 15

2. **Violin/Box Plot**:
    - This section provides a visual representation of the gene set scores using violin or box plots.
    - Select the X-axis (e.g., "time") and Y-axis (e.g., "nCount_Spatial") parameters.
    - Choose the plot type (violin or boxplot).
    - Toggle between showing data points and adjusting graphics controls.
    - Download the plots as PDF or PNG files.



GeneSet Score violin plot / box plot

Spot information (X-axis): time

Spot Info / GeneSet name (Y-axis): nCount_Spatial

Plot type: ● violin ○ boxplot

☐ Show data points

PDF / PNG height: 8

PDF / PNG width: 10

How to Use Data Download Tab

The Data Download tab in the STOP-CKD database provides users with access to various datasets and resources related to chronic kidney disease research. This includes processed data, visualizations, and supporting materials such as videos. Here’s a detailed guide on how to use this feature effectively:

Accessing the Data Download Tab

1. **Select the Data Download Tab**: Click on the "Data Download" tab on the top navigation bar to open the data download interface.

Downloading Datasets

1. **Available Data Types**:
    - The Data Download tab offers a variety of data types that can be downloaded for further analysis. These include:
        - **Processed Expression Matrices**: Provides processed data with gene expression levels across different conditions and regions.
        - **Metadata**: Contains detailed information about the samples, experimental conditions, and processing steps.
        - **Gene Set Scores**: Gene list for various gene sets, useful for pathway and functional analysis.
2. **Selecting Data for Download**:
    - Browse through the list of available datasets and select the ones you are interested in.
3. **Initiating Download**:
    - Click on the download link or button next to the dataset you wish to download.
    - Depending on the file size and your internet connection, the download may take some time.