

Frequently Asked Questions

- **General**
 - How to create a project?
 - Can I change my user avatar?
 - How do I add and use my own lists?
 - Can I repeat a task and everything downstream of it, while changing only one/a few parameters?
 - What can I use to identify cells that are actively expressing genes within a gene list?
 - Can I build and use pipelines for my analysis?
 - How do I classify cells?
 - My server is full, how do I make more space?
 - How do I add library files if I am not studying human or mouse?
 - Are Genome coordinates 1-based or 0-based?
 - Can I add transgenes to my reference files?
 - How do I export data from the result nodes?
- **Visualization**
 - How do I order my heatmap by the cell types?
 - How do I display UMAP for each sample in the Data Viewer?
 - Can I visualize fold change values on a heatmap without using a z-score?
 - Why don't I see Flip mode on the heatmap? Why can't I download all of the data after zooming?
 - How to label gene names on volcano plot?
- **Statistics**
 - Why do I get "?" for FDR p-values in my Deseq2 result?
 - What is fold change?
 - Can I label a Volcano plot with gene names?
 - In Volcano plot, what is inconclusive group mean?
 - What is the difference between FDR and FDR step up?
 - How to perform a paired t-Test in Flow
 - Can I compare one attribute at a time versus all of the others combined?
 - I downloaded gene counts from the output data node generated by the Quantify to annotation model task, why can't I find my genes of interest?
- **Biological Interpretation**
 - What is the difference between GSEA and Gene Set Enrichment?
 - What is the enrichment score shown in the Gene Set Enrichment report?
 - In KEGG pathway, genes can be colored by Fold change and p-value etc, how are the gene statistics calculated?
 - When should I use GSEA or Gene set ANOVA?

General

How to create a project?

To create a project, you first need to [transfer files to the Partek Flow server](#), and then import the files into your project using the import data wizard, here is the [video](#) and [more information](#).

Can I change my user avatar?

Yes, navigate to [My profile](#) and click the "Change image" button. Do this by clicking your avatar at the top right corner of the interface, select [Settings](#), then choose [Profile](#).

How do I add and use my own lists?

Click your avatar in the top right corner of the Partek Flow interface, choose [Settings](#) in the menu, and select [Lists](#) from the left panel of the [Components](#) section. Lists can also be generated from result tables using the "Save as managed list" button. For more information please click [here](#).

Can I repeat a task and everything downstream of it, while changing only one/a few parameters?

Yes, click on the rectangular task that you want to change the parameters. On the context-specific menu on the right, under Task actions, select 'Rerun with downstream tasks', this will bring you to the task set up page where you can edit the parameters for the task, then click Finish to run the task with the new parameters. The tasks downstream of it will be initiated automatically.

What can I use to identify cells that are actively expressing genes within a gene list?

Use [AUCell](#) to identify cells with active gene sets; this task calculates a value for each cell by ranking all genes by their expression level in the cell and identifying what proportion of the genes from the gene list fall within the top 5% (default cutoff) of genes. An alternative option is to use the *Gene score* for a feature list to select and filter populations based on the distribution; [click here for more information](#).

Can I build and use pipelines for my analysis?

Yes, click on [Import a pipeline](#) on the bottom of the [Analyses](#) tab dashboard. This will help you import either our hosted pipelines or your own saved pipeline which can be found under [Settings](#) -> [Components](#) -> [Pipelines](#). Click [here](#) for steps to save and run a pipeline. For more information related to navigating pipelines click [here](#).

How do I classify cells?

Classification in Partek Flow can be performed manually or with automatic cell classification which is explained in more detail [here](#). Users often want to classify cells by gene expression threshold(s), for details on classification by marker expression click [here](#). Automatic classification needs to be performed on a non-normalized single cell data node; once complete, [publish cell attributes to project](#) then use this classification in visualizations and tasks. You may choose to perform [Graph-based clustering](#) and [K-means clustering](#) to help identify biomarkers that can then be used to identify the clusters and we also provide hosted lists for different cell types.

My server is full, how do I make more space?

We recommend cleaning up projects as well as removing library files that you do not need, then removing the orphaned files. You can also export analyzed projects and save them on an external machine, then when you need them again you can import them to the server. Please see this information for more details related to: [Project management](#), [Removing library files](#), and [Orphaned files](#). Right click on the data node to delete files from projects that are not needed (e.g. fastqs from project pipelines that are analyzed); you will not be able to perform tasks from this node once the files are deleted.

How do I add library files if I am not studying human or mouse?

To add a new assembly, click on [Settings](#) -> [Library files](#). From the Assembly drop-down list, select *Add assembly* and specify the species. If the species name is not in the list, choose *Other* and type in the name with the assembly version (multiple assembly versions can exist for one species, e.g. hg19 and hg38 for Homo Sapiens). You need to add the reference file which is a .fasta file containing sequence information. Once the reference file is added, you can build any aligner index to perform the alignment task.

The Annotation model is a file containing feature location. This file can be used to quantify to annotation model in RNA-Seq analysis, or annotate variant or peaks in a DNA-Seq or ATAC-Seq/ChIP-Seq data analysis pipeline. The file format should be .gtf/.gff/.bed.

We recommend looking for the species files on the [Ensembl](#) website. There is no need to unzip or save these files to your local machine, instead right click and copy the link address of the specific file (not a link to a folder). For more details, here is the documentation chapter: [Library File Management - Partek® Documentation](#).

Are Genome coordinates 1-based or 0-based?

Genome coordinates for annotation models stored in Partek Flow are 1-based, start-inclusive, and stop-exclusive. This means that the first base position starts from one, the start coordinate for a feature is included in the feature and the stop/end coordinate is not included in the feature. These are the genome coordinates that are printed in various task reports and output files when an annotation model is involved in the task. When custom annotation files are added to Partek Flow, the genome coordinates are converted into this format. The coordinates are converted back if necessary for a specific task. shows how the genome coordinates vary between different annotation formats.

Format	1- or 0-based	Start	Stop/End	Example (Start, Stop/End)
GTF/GFF/GFF3	1	inclusive	inclusive	1000, 2000
BED	0	inclusive	exclusive	999, 2000
Partek	1	inclusive	exclusive	1000, 2001

Can I add transgenes to my reference files?

Yes, to add transgenes (including gfp or related) to the references files, first choose an assembly, create the transgene reference, and merge the references together (e.g. combine mm10 with dttomato). This is the same process for the annotation file.

How do I export data from the result nodes?

Left click to select the data node you want to export. In the bottom of the task menu there will be an option to [Download data](#).

Visualization

How do I order my heatmap by the cell types?

If you would like specific groups (e.g. cell types) in a certain order, do not perform [Hierarchical clustering](#) on these cells and instead choose to assign order, then use click and drag to reorder the groups. If you want to remove a group, you can choose to exclude this group in the filtering section. You can still perform [Hierarchical clustering](#) on the features if you would like to. Hierarchical clustering will force the heatmap to cluster and you would need to click the dendrogram nodes to switch the order. Click [here](#) for more information.

How do I display UMAP for each sample in the Data Viewer?

For a multi-sample project, all of the downstream tasks will be run separately if 'Split by sample' was checked when performing the PCA task. Visualization of different samples can be displayed by 'Sample' using the 'Misc' section in the [Axes](#) card. To show different samples side by side, one can click 'Duplicate plot' first, then use the 'Sample' option to switch the samples.

Can I visualize fold change values on a heatmap without using a z-score?

Yes, the default settings can be modified by clicking "Configure" in the Advanced settings during task set-up, then change the "feature scaling" option to "none" to plot the values without scaling. For more information related to the heatmap click [here](#).

Why don't I see Flip mode on the heatmap? Why can't I download all of the data after zooming?

The Flip mode and download all data options are disabled if there are more than 2.5 million values (rows x columns) in the heatmap.

How to label gene names on volcano plot?

By default, genes are selected if the p-value is ≤ 0.05 and $|\text{fold change}| \geq 2$ and when the number of selected genes is less than 2000 genes, they will be labeled. You can click on **Style** button in *Configure* section, choose a gene annotation field from the *Label by* drop-down list to change the label. If you number of selected genes is select less than or equal to 100, Partek Flow will try to spread out labels as much as possible to clearly display the labels. If number of selected genes is more than 100, labels will be next to the selected genes, there will be overlaps where genes are close together. If there are more than 2000 genes selected, no label will be displayed.

If you click any blank space, you can turn off select and use different selection mode button on the vertical bar on the upper-right corner of the plot to manually select dots on the plot.

Statistics

Why do I get "?" for FDR p-values in my Deseq2 result?

When a feature (gene) has low expression, it will be filtered by automatic independent filtering. To avoid this, you can either [filter features](#) to exclude low expression features before Deseq2, or in the Deseq2 advanced options, choose [apply independent filtering](#) setting. Details about independent filtering can be found at the [Deseq2 documentation](#).

[Click here for troubleshooting other differential analysis models and "?" results](#)

What is fold change?

Fold change indicates the extent of increase or decrease in feature expression in a comparison. In Partek Flow, fold change is in linear scale (even if the input data is in log scale). It is converted from ratio, which is the LSmean of group one divided by LSmean of group two in your comparison. When the ratio is greater than 1, fold change is identical to ratio; when the ratio is less than 1, fold change is $-1/\text{ratio}$. There is no fold change value between -1 to 1. When ratio/fold change is 1, that means there is no change between the two groups.

Log ratio option in Partek Flow is converted from ratio, this is a value comparable to log fold change in some other tools.

Can I label a Volcano plot with gene names?

Yes, go to Style in the [Data Viewer](#) and make sure Gene name is selected under "Labeling". Next, go to the in plot selection tools (right side of the graphic) and use any of the selection tools to select the cells that you would like to label. You can use ctrl or shift to select multiple populations at once. For more information on the Volcano plot click [here](#).

In Volcano plot, what is inconclusive group mean?

By default, Flow is using the p value ≤ 0.05 and $|\text{fold change}| \geq 2$ as the significance cutoff. If genes meet both p value and fold change cutoff, they are significantly up or down regulated genes. If they only meet one criteria, they are called inconclusive. If genes won't pass either criteria, they are not significant. Click on the **Statistics** button in the *Configure* section in the left control panel, you can change the cutoff. Click on the **Style** button to change the color of significance categories.

What is the difference between FDR and FDR step up?

FDR is the expected proportion of false discoveries among all discoveries. FDR Step-up is a particular method to keep FDR under a given level, alpha, that was proposed in this [paper](#). In Partek Flow, if one calls all of the features with p-values 0.02 or less, the FDR is less or equal to 0.41.

How to perform a paired t-Test in Flow

You should have at least the following two attributes in the Metadata, treatment (including two subgroups) and subject ID (to pair the two samples). When performing differential analysis, choose ANOVA and include both attributes into the ANOVA model, the two-way ANOVA is mathematically equivalent to paired t-Test.

Can I compare one attribute at a time versus all of the others combined?

Yes, you can use the [Compute biomarkers](#) task to compare one subgroup at a time to all of the others combined. An alternative option is to set up the differential analysis model in this way; for more information please see the information [here](#) for each model.

I downloaded gene counts from the output data node generated by the Quantify to annotation model task, why can't I find my genes of interest?

In the [Quantifying to an annotation model](#) dialog, by default, Partek Flow filters features based on the total count across all of the samples and features with a total count greater than 10 will be reported. If you want to report all of the genes in the annotation file, change the *Filter features* value to **0**.

Biological Interpretation

What is the difference between GSEA and Gene Set Enrichment?

In Partek Flow, GSEA should be performed on a sample/cell and feature matrix data node (e.g. normalization count data). GSEA is used to detect a gene set/a pathway which is significantly different between two groups. Gene set enrichment should be performed on a filtered gene list; it is used to identify overrepresented gene set/pathway based the filtered gene list using Fisher's exact test. The input data is a filtered list using gene names.

What is the enrichment score shown in the Gene Set Enrichment report?

The enrichment score shown in the enrichment report is the negative natural log of the enrichment p-value derived from Fisher Exact test. The higher the enrichment score, the more overrepresented our list of genes in the gene set of a GO/pathway category.

In KEGG pathway, genes can be colored by Fold change and p-value etc, how are the gene statistics calculated?

For Gene set enrichment analysis, only genes from the input data node (filtered gene list) will be colored in the KEGG pathway gene network, using the statistics in the data node.

During GSEA (or Gene set ANOVA) computation, we also perform ANOVA on each gene based on the attributed selected independent from GSEA computation (at gene set level). The results of ANOVA is only used to color the genes in the KEGG gene network. If GSEA is computed using another other database, e.g. GO, we don't compute ANOVA on each gene since GO databased doesn't have gene network information.

When should I use GSEA or Gene set ANOVA?

Both methods should be performed on a normalized matrix data node, and requires gene symbol in feature annotation. Both methods are detecting a differentially expressed Gene set (pathway) instead of each individual gene. The algorithms are different. GSEA is a popular method from the [Broad institute](#). Gene Set ANOVA is based on generalized linear model, [here](#) are the details.