Mapping Pathways and Ontologies for PamChip® data using g:Profiler, a free online tool

Phosphosites on the PamChip (PTK and STK) can be phosphorylated by a number of kinases. The Upstream Kinase Tool is a functional scoring method that ranks the Top kinases differentially activated comparing 2 conditions. Pathway and Ontology analysis of phosphosite level data and/or kinase level data is useful for further biological interpretation

Phosphosite Level data:

Comparative analyses using T-tests or ANOVA-Dunnett tests, result in Tables with Log2Fold Change (LFC) and p-values for each Test Condition compared to Control (T vs C), per Phosphosite (UniPROT ID) for each of the Protein Tyrosine Kinase (PTK) and Ser/Thr Kinase (STK) assays. Data extracted from the Tables are used for Enrichr analysis.

- If applicable, combine the PTK and STK Tables

- Select a p-value threshold (recommended <0.05), to select the significant UP or DOWN phosphosites

- Order the Table by LFC, to separate the UP and DOWN regulated Phosphosite UniPROT IDs

Kinase Level data:

Kinase Score Tables are generated from PamChip Data (PamApp Tool, Upstream Kinase Analysis/UKA), for each comparison, for each assay. The prediction of Top kinases differentially activated between 2 conditions can provide biological insights, especially when analyzed in the context of ontologies and pathways. Data extracted from the Tables are used for Enrichr analysis.

- If applicable, combine the PTK and STK Tables

- Select a threshold cut-off (recommended Median Final Score >1.2)

- Order the Table by Median Kinase Statistic, to separate the UP and DOWN regulated Kinase UniPROT IDs

Combining data:

- If applicable, for each comparison, combine the pre-selected, significantly UP or DOWN (or both) regulated UniPROT IDs from the Phosphosite level and/or Kinase level data

- Make a list of either UP or DOWN UniPROT IDs (or both) per comparison.

Data analysis with g:Profiler

- Pathway or Gene Ontology analysis can be done using g:Profiler (https://biit.cs.ut.ee/gprofiler/gost)

- The UP or DOWN (or both) lists of the UniPROT IDs (or Gene names) for each comparison, can be copy-pasted to **g;Profiler** space where indicated

- From the pulldown menus on the right, select your advance options, data sources etc.

- Pressing the Run query button will result in several Results TABs that you explore. **Run query**

- The ResultsTABs can provide interesting data from different tools/ sources, that can be customized (choose the analysis or resolution you want) downloaded and saved as png or csv files for Figures or Tables.

- G:Profiler looks at combinations of genes and aims to identify which pathways are differentially activated. You can look at each pathway image by looking them up in the KEGG database (https://www.kegg.jp/kegg/pathway.html).