**Partek Flow Training**

**RNA-seq**

**Login and project setup**

1. Open Google Chrome and please enter URL: **demo.partek.com**
2. Login as your **given** “username” and “password”
3. Click “**New project**” and enter project name: RNAseq-[username], e.g.: RNAseq-user1
4. Click “**Add samples**” > “**Automatically create samples from files**”
5. Browse to **/home/flow/FlowData/RNA\_Seq/** and add all samples in the folder.
6. Click “**Assign sample attributes from a file**”
7. Browse to **/home/flow/FlowData/RNA\_seq/** select “**sampleInfo.txt**” and click “**Next**” and then “**Import**”.

**Analyze the data**

1. Go to “**Analyses**” tab
2. Pre-alignment QA/QC (Evaluating the quality of raw reads)
   1. Select “**Unaligned reads**” data node
   2. Click “**Pre-alignment QA/QC**” from the “**QA/QC**” section in the toolbox
   3. Use default settings, and click “**Finish**”.
3. Pre-analysis tools (Pre-alignment action on raw data)
   1. Select “**Unaligned reads**” data node
   2. Click “**Trim** **bases**” from the “**Pre-analysis tools**” section in the toolbox
   3. Select “Tri**m based on: Quality score**” with default settings and click “**Finish**”
4. Alignment (Aligning the data to the reference sequences)
   1. Select “**Trimmed reads**” data node
   2. Click “**STAR**” from the “**Aligner**” section in the toolbox
   3. Select STAR index:

* Genome build: **Homo sapiens (human) - hg19\_chr22**
* Index: **Whole genome**
  1. Use the default options, click “**Finish”**

1. Post-alignment QA/QC (Evaluating the quality of alignment)
   1. Select “**Aligned reads**” data node
   2. Click “**Post-alignment QA/QC**” from the “**QA/QC**” section in the toolbox
   3. Use default settings and click “**Finish**”
2. mRNA Quantification (mapping aligned reads to transcriptome database)
   1. Select “**Aligned reads**” data node
   2. Click “**Quantify to transcriptome (E/M)**” from the “**RNA-Seq Analysis**” section
   3. Select “**Refseq**” as the “**Annotation model**” and click “**Finish**”
3. Quantification report and PCA plot
   1. Select “**Quantification**” data node
   2. Click “**Task report**” in the toolbox
   3. Click “**Download gene-level read counts”**, to save it as text file
4. Differential expression analysis
   1. Select “**Quantification**” data node
   2. Click “**Differential gene expression**” from the “**RNA-Seq Analysis**” section
   3. Select “**5uM vs 0uM**” and click “**Add comparison**”
   4. Select “**10uM vs 0uM**” and click “**Add comparison**”
   5. Select “**Run analysis on Gene-level**” and click “**Finish**”

**Visualizing Results**

1. View Differential expression analysis results
   * 1. Select “**Feature List”** data node and then click “**Task report**” in the toolbox
     2. Under the “**Gene list**” section, on the “**Filtering**” panel select “**FDR step up**”, then select “**All contrasts**” and set it to **Less than or equal to** **0.05**
     3. Under the “**Gene list**” section, on the “**Filtering**” panel select “Fold-change”, then select “**All contrasts**” and set it to From “**-2 to 2**”, then check “**Exclude range**”
     4. On the gene-list table, under the view column, select  to get the **Dot plot**
     5. On the gene-list table, under the view column, select  to see in **Chromosome View**
     6. On the gene-list table, under the view column, select  to get the GSA extra details report for the gene
     7. At the bottom of the table, click  to generate a new “**Feature List**”
2. Hierarchical Clustering
3. Select “**Feature List**” data node after *Filtered gene analysis* task
4. Click “**Hierarchical clustering**” from the “**RNA-Seq Analysis**”
5. Use default parameters and click “**Finish**”
6. Select the “**Hierarchical clustering**” task node and click on “**Task Report**”

**Biological Interpretation**

1. Select “**Feature List**” data node after *Filtered gene analysis* task
2. Select “**Enrichment analysis**” from the “**Biological interpretation**”
3. Select “**GO**” as Gene set annotation and then click on “**Finish**”
4. Select the “**Enrichment**” task node and click on “**Task Report**”

**Creating pipelines**

1. On Analysis tab, click “**Make a pipeline”** at the lower-left of the page
   1. Name the pipeline as “RNAseq-Pipeline-[username]”
   2. Select “**Section name: Pipelines**” then select the task nodes (rectangles) to include in the pipeline
   3. Click “**Make pipeline**” to create the pipeline

**DNA-seq**

**Import bam files**

1. Click “**New project**” and enter project name: DNAseq-[username], e.g.: DNAseq-user1
2. Click “**Add samples**” > “**Automatically create samples from files**”
3. Browse to **/home/flow/FlowData/DNA\_Seq/** and add samples in the folder.

**Remove duplicated reads**

1. Select **Aligned reads** data node, choose **Filter bam files task** inPost alignment toolssection, set **Max reads per genomic position** to **1**
2. Check **compare sequence** and **Keep alignment with highest mapping score,** click **Finish**

**Variant detection**

1. Select **Detect variants against reference**, choose **Partek** method and Genome build is **hg19\_chr22**, leave option set as default, click **Finish**
2. Select **Variants** data node and choose perform **View variants**
3. Select **Variants** data node and choose **Annotate with known variants > dbSNP and Annotate with genomic features > Refseq,** click **Finish**
4. Select **Annotated variants** data node, choose **Filter variant:**
5. Select the result data node, choose **View variants**
6. View the result in chromosome view

**Visualization**

1. Select tracks:
   1. Click on the enabled data node, check the available tracks to show
   2. Check to add annotation track
2. Controls:
   1. Change the color to represent base
   2. In selection mode, click on a position in histogram track to show the read counts of each base
   3. In selection mode, click on a position in pipe-up track to show read detailed information
   4. In selection mode, click on a position in variant track to show base frequency
   5. In selection mode, click on a annotation track to see refseqID and gene name