### **Partek Flow FAQs**

Here are some Frequently Asked Questions about Partek Flow software based on real support calls.

### Why did my import fail?

The most common form of failure is truncation. Ensure the transfer is complete before importing the data and that the file size and MD5 reported by Flow match the original data.

For bam files make sure the SAMtools utility can sort and index the file.

For fastq files run gunzip -c filename | tail and look for the message "unexpected end of file" or a missing component of the last read.

#### Why can't I find the RPKM method on the normalization menu (I see FPKM)?

When working with paired data it should be the case that FPKM is available, and when working with single end data RPKM should be available. These metrics are essentially analogous, but based on the underlying method used for calculation (accounting for two reads mapping to 1 fragment and not counting twice for paired end data). Here is a simple description of the differences in calculation between RPKM and FPKM: <a href="http://www.rna-seqblog.com/rpkm-fpkm-and-tpm-clearly-explained">http://www.rna-seqblog.com</a> /rpkm-fpkm-and-tpm-clearly-explained.

#### Why don't you have RPM in your normalization?

RPM (reads per million) is the same as Total Count. Please use Total Count.

#### What is a canonical transcript?

For genes with multiple transcripts, one of the transcripts is picked as the canonical transcript. Based on the UCSC definition from the table browser,

knownCanonical - identifies the canonical isoform of each cluster ID, or gene. Generally, this is the longest isoform.

we define the canonical transcript as either the longest CDS (coding DNA sequence) if the gene has translated transcripts, or the longest cDNA.

#### Why are there decimal values in the Partek E/M quantification output?

The Partek E/M quantification algorithm can give decimal values because of multi-mapping reads (the same read potentially aligning to multiple locations) and overlapping transcripts/genes (a read that maps to a location with multiple transcripts or genes at that location). In these scenarios, the read count will be split.

For example, if a read maps to two potential locations, then that read contributes 0.5 counts to the first location and 0.5 counts to the second location. Similarly, if a read maps to one location with two overlapping genes, then that read contributes 0.5 counts to the first gene and 0.5 counts to the second gene.

If you need to remove the decimal points for downstream analysis outside of Partek Flow, you can round the values to the nearest integer.

#### Why can't I view PCA on Google Chrome and I am being pointed to WebGL instructions?

If you are using Google Chrome, some graphics card (particularly in older Mac laptops) are **not supported** and they affect the ability to run WebGL (needed to display PCA). You may wish to use a different browser (Mozilla Firefox) or you can try running Google Chrome while ignoring any blacklisted hardware:

- For Mac, this is the command: /Applications/Google\ Chrome.app/Contents/MacOS/Google\ Chrome --ignore-gpu-blacklist
- Here is a video of a workaround and also creating an automated tool in a Mac: https://customer.partek.com/tutorials/ChromeWebGLWorkaround. mp4 (02:55)
- The app created in the video is available for download here (ChromeWebGL.app.zip). Unzip and make sure to exit Google Chrome complete before running the app.

## I have two projects that I ran separately. I want to combine the two projects without repeating the alignment step. How can I combine the aligned reads?

Create a third project. Import two sets of BAM files.

Project 1 - In Data Tab, take note of your project output folder. Also note the aligner that you used.

Project 2 - In Data Tab, take note of your project output folder. Also note the aligner that you used.

Project 3 - Create project. Import Data > Automatically create samples from files > Find the project output folder of Project 1 (should be in Partek Flow server) > Find the folder for the aligner you used > Within that folder should be the BAM files > Select the BAM files > Create sample. Repeat same process for Project 2.

Now you will have all samples as one data node (aligned reads) in Project 3. You can proceed with downstream analysis (normalization, differential gene expression, etc).

# Why is the number of variants listed in the variant reports and summarize cohort mutations report different?

For variants with multiple alternative alleles, the variant has one row for all alternative alleles, while the summarize cohort mutations report lists each alternative allele on a separate rows. The number of variants listed at the top of the each report is calculated from the number of rows in the report.

#### Additional Assistance

If you need additional assistance, please visit our support page to submit a help ticket or find phone numbers for regional support.

