**Using** safeQuant.RUpdated 2015-03-13

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**Basic usage common to all workflows**

1) Navigate to the folder where the safeQuant.R script is installed.

bz-wg2-pll01:exec erikahrne$ cd /Users/erikahrne/dev/R/workspace/SafeQuant/exec

2) Display help

bz-wg2-pll01:exec erikahrne$ Rscript safeQuant.R -h

3) Specify input file

bz-wg2-pll01:exec erikahrne$ Rscript safeQuant.R -i ../inst/testData/QI\_2.0/peptide\_measurements1.csv

4) Define experimental design

*2 Conditions. Control Samples: 1,2,3. Case 1: Samples 4,5*

bz-wg2-pll01:exec erikahrne$ Rscript safeQuant.R -i ../inst/testData/QI\_2.0/peptide\_measurements2.csv --EX 1,2,3:4,5

*3 Conditions. Control Samples: 4,5. Case 1: Samples 1,2. Case 2: Sample 3*

bz-wg2-pll01:exec erikahrne$ Rscript safeQuant.R -i ../inst/testData/QI\_2.0/peptide\_measurements2.csv --EX 4,5:1,2:3

*2 Conditions. Control Samples: 4,5. Case 1: Sample 1*

bz-wg2-pll01:exec erikahrne$ Rscript safeQuant.R -i ../inst/testData/QI\_2.0/peptide\_measurements2.csv --EX 4,5:1

5) Require at least X peptide per protein

bz-wg2-pll01:exec erikahrne$ Rscript safeQuant.R -i ../inst/testData/QI\_2.0/peptide\_measurements2.csv --FN 2

**LFQ Protein Relative Quantification**

Compatible with Feature, Protein and Peptide Measurement Progenesis .csv exports. If using Progenesis QI 2.0 or later we recommend using Peptide Measurement Progenesis exports.

To export:

- File -> Export Peptide Measurements. This option is available once you have reached the "Resolve Conflicts" Step in Progenesis QI.

- When choosing properties to be included in the exported file check the "All accessions (for this sequence)" check box.

**LFQ Phospho Relative Quantification**

bz-wg2-pll01:exec erikahrne$ Rscript safeQuant.R -i ../inst/testData/2014/peptides1\_FILTERED.csv -f ../inst/testData/2014/sp\_hum\_160512.decoy.fasta --FM phospho –-EP –FS 2

-f ../inst/testData/2014/sp\_hum\_160512.decoy.fasta

Supply protein (.fasta) database so that all phospho sites will be annotated with their protein position (amino acid number starting from n-term) as well as sequence motifs (motif-X input format <http://motif-x.med.harvard.edu/>).

--FM phospho

Filter data to only include phospho peptides

--EP

Do not carry out Protein level quantification

--FS 2

Filter data to only include peptides carrying maximum 2 variable PTMs.

**TMT Protein Relative Quantification**

bz-wg2-pll01:exec erikahrne$ Rscript safeQuant.R -i ../inst/testData/2014/TMT\_10-Plex\_Scaffold\_Raw\_Export\_Example.xls

Make sure to specifiy the experimental design if needed using (--EX flag).

TMT 6-plex default experimental design is 1,2,3:4,5,6

TMT 10-plex default experimental design is 1,2,3,4:5,6,7:8,9,10

**LFQ Protein Absolute Abundance Estimation**

Calculate intensity-based absolute-protein-quantification (iBAQ) metric per protein.

Reference: Global quantification of mammalian gene expression control, Schwanhausser (2011), \url{http://www.ncbi.nlm.nih.gov/pubmed/21593866}

bz-wg2-pll01:exec erikahrne$ Rscript safeQuant.R -i ../inst/testData/2014/peptides2\_FILTERED.csv -f ../inst/testData/2014/sp\_mouse\_160512.decoy.fasta –AI

Calculate Top3 (Mean of X most intense features)

Reference: Absolute quantification of proteins by LCMSE: A virtue of parallel MS acquisition, Silva (2006), \url{http://www.ncbi.nlm.nih.gov/pubmed/16219938}

bz-wg2-pll01:exec erikahrne$ Rscript safeQuant.R -i ../inst/testData/2014/peptides2\_FILTERED.csv --AT