Quantification of peptides in MS-based proteomics

Introduction

In this note, we describe how to use the MS peptide quant application on the Lunatic systems. This application is used for quantification of proteomic peptide samples (µg/µL) derived from complex protein mixtures with reporting of desired injection volume for a LC-MS/MS system.

App selection

On the Big Lunatic, the MS peptide quant application can be found under the "Protein" sample type button in the "Classic" column (Figure 1). On the Little Lunatic, this application can be found on the applications screen (Figure 2). For proper use of the application, always use the sample solution buffer as blank(s). Aside from sample names, additional user input can be added:

Sample volume (µL): available sample volume

Injection amount (µg): desired amount of peptide material to be injected on the LC-MS/MS system

Results on screen

On the Big and Little Lunatic, injection volume values are shown in the overview tab and the slide thumbnail view respectively. For each sample, a more detailed analysis can be found in the Big Lunatic's details tab and below the graph on the Little Lunatic (Figures 3 and 4):

- **Injection volume**: the injection volume is calculated based on the user defined injection amount and the measured peptide concentration (injection amount (µg)/peptide concentration (µg/µL)= injection volume (µL)).
- **Peptide concentration**: the baseline corrected UV/Vis spectrum (black curve on the Big Lunatic, white curve on the Little Lunatic) is used to calculate the peptide concentration in µg/µL. This is done using the absorbance values at 280 nm

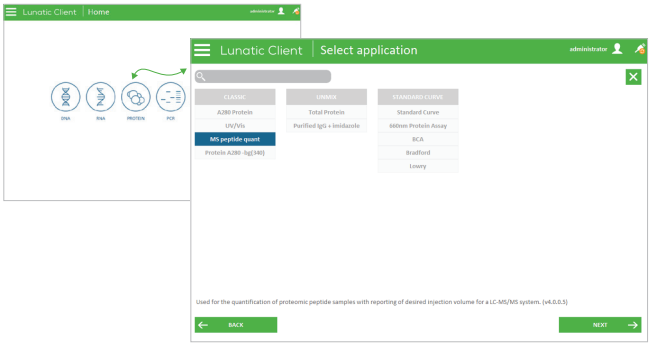


Figure 1: Illustration of the Big Lunatic interface. The image in the back shows the Sample Type screen whereas the image in the front displays the available applications for the selected Sample Type.

Figure 2: App button on the Little Lunatic app selection screen.

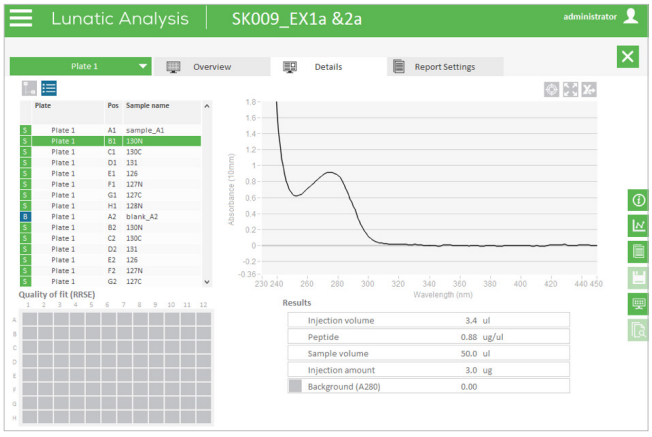


Figure 3: Illustration of the Results screen on the Big Lunatic. In addition to the injection volume, the peptide concentration is also displayed.

and a default E1% percent extinction coefficient =10 for complex peptide mixtures.

- **Background (gray)**: sample turbidity profile. The background spectrum is subtracted from the measured spectrum, resulting in the content spectrum.

A horizontal gray band on the spectrum indicates saturation when absorbance values have passed the upper limit of detection (275 OD on a High Lunatic Plate or Chip). Values outside the linear range are not reliable.

Report

A variety of report types are generated: an HTML, XML, TXT and a CSV file are created on both systems. In addition, the Big Lunatic also creates XLSX and PDF report files. On the Little Lunatic fixed report templates are used while the Big Lunatic allows full flexible selection of the content to be reported.

Case Study

In order to investigate the optimal peptide amount to be injected, a dilution series of a K-562 cellular proteome digest (Promega, V6951; 0.25 – 9 µg) was injected for LC-MS/MS analysis on an Ultimate 3000 RSLCnano System, in-line connected to a Q Exactive HF mass spectrometer (Thermo). Peptides were separated on a 40 cm nano-LC column (1.9 µm C18 beads) by an increase in acetonitrile concentration over 140 minutes. This data suggests 3 to 6 µg as an optimal peptide amount to be loaded on this LC-MS/MS setup. Peptide identifications start dropping again when injecting more material (Figure 5A). Injection of a typical amount, ranging from 250 ng up to 2 µg, only reveals a fraction of the proteome indicating that injection of a more optimal amount can boost peptide and thus protein identifications. In order to further narrow down the optimal peptide injection amount for the LC-MS/MS setup used, the average peptide chromatographic peak area in all samples was analyzed (Figure 5B). These results point to 3 µg as the optimal amount.

Not only at the LC level, but also at the MS level the effect of an optimal peptide injection amount could be observed (Figure 5C). Plotting the average MS2 injection time clearly shows shorter injection times

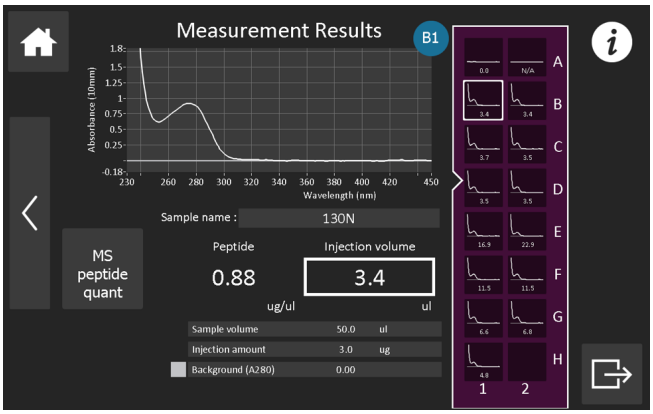
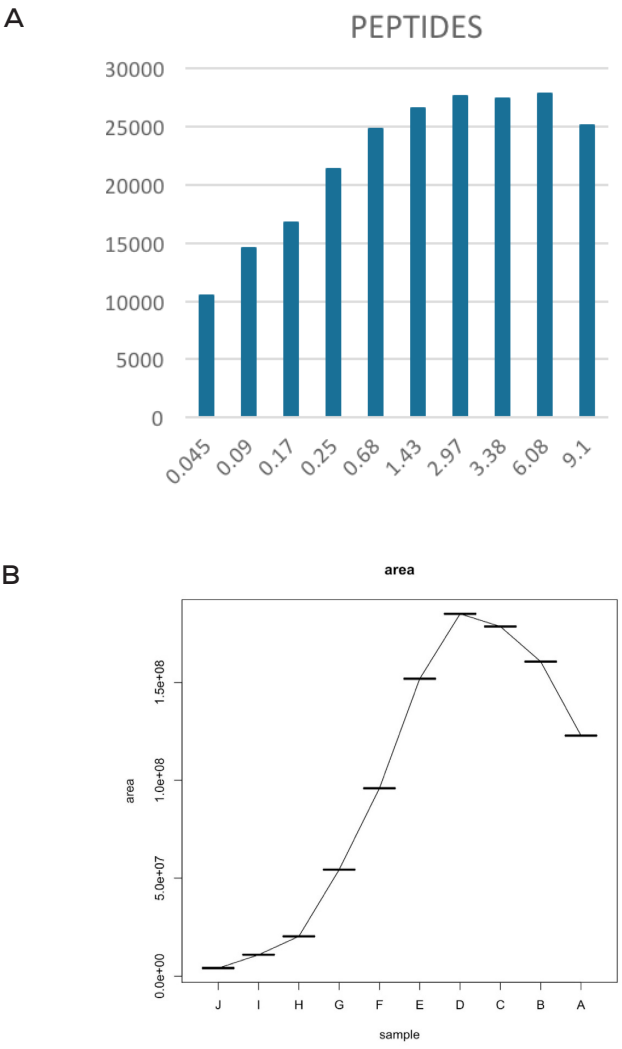


Figure 4: Illustration of the Results screen on the Little Lunatic. In addition to the injection volume, the peptide concentration is also displayed.



when getting closer to the optimal injection amount of 3 µg of peptides, the same optimum found by chromatographic peak area analysis.

C

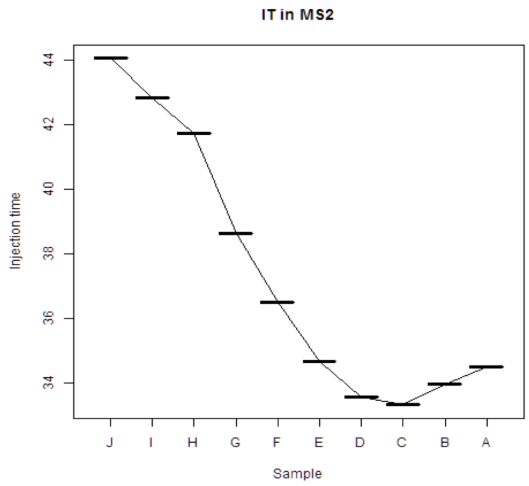


Figure 5: Peptide identification and concentration correlation. (A) A histogram plotting the number of peptides identified by LC-MS/MS analysis for a dilution series of a K-562 cellular proteome digest. (B) Average peptide chromatographic peak area. (C) Average MS2 injection time.

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