MOLECULAR SEROLOGY PROCEDURES MANUAL

Body Fluid Identification by Proteomic Mass Spectrometry -Analysis		
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# Body Fluid Identification by Proteomic Mass Spectrometry - Analysis

1 Purpose

1.1 Process data accumulated through the use of the liquid chromatographer and mass spectrometer in order to identify presence of specific body fluids on evidence samples using Skyline and R Script. Skyline is an open-source software for targeted proteomics data analysis.
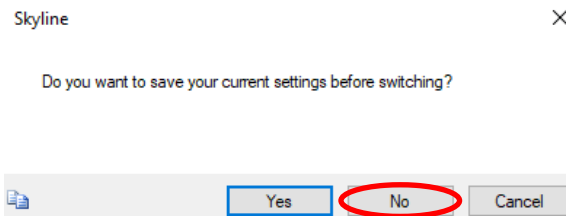
2 Skyline Analysis Procedure

2.1 Double-click on Skyline software on desktop.

2.2 On the Start Page click on Blank Document
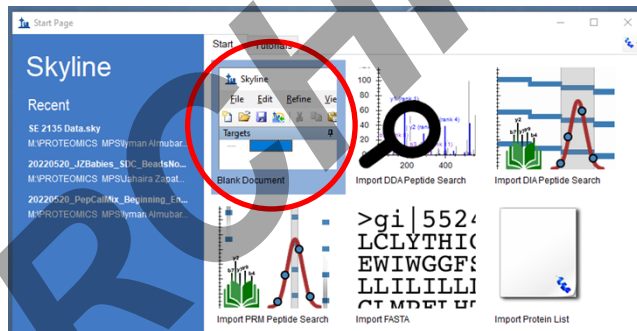
2.3 Click Settings → OCME Serology

2.4 Click No.

2.5 Click Settings → Peptide Settings → Modifications and ensure "Internal standard type" to "None". If not, set to "None". Click OK.

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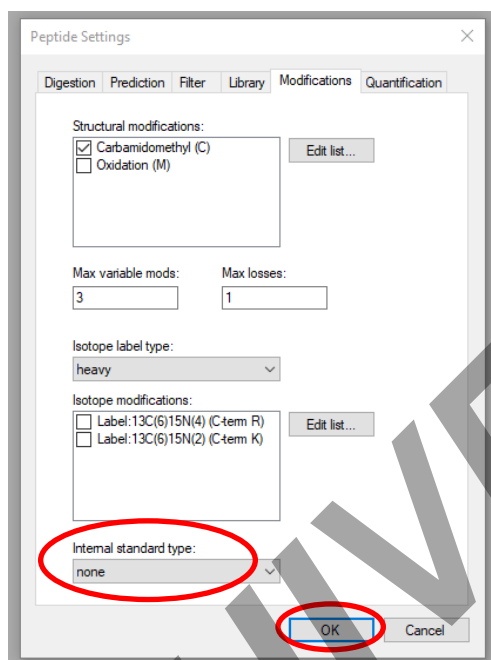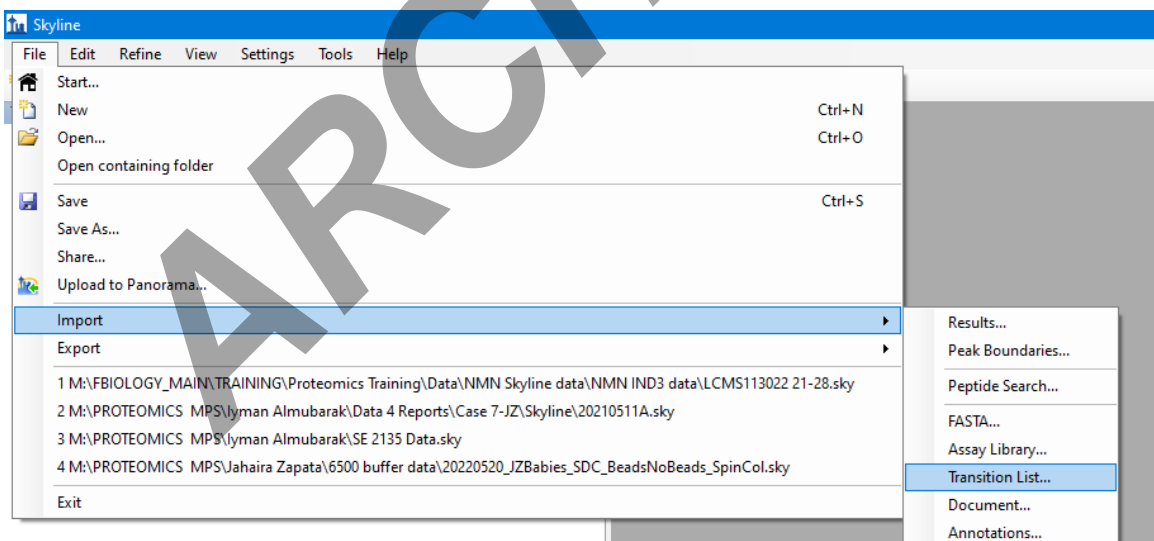| Body Fluid Identification by Proteomic Mass Spectrometry -Analysis		
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2.6   Select File → Import → Transition List

<mark>2.7   Open the excel file labeled "Final B SE and SA transitions with Cyto C" **found in "Molecular Serology_Data" folder on FBiology drive**</mark>

2.8   Ensure "peptides" is selected. Click OK.

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2.9 Click File → Save As → Rename File as DATE (YYYYMMDD)_Batch X, where X is A, B, C, etc in the order in which the batch is run on each date. Save file in "Molecular Serology Data" folder on FBiology drive.

2.10 Select File → Import → Results

2.11 Click OK

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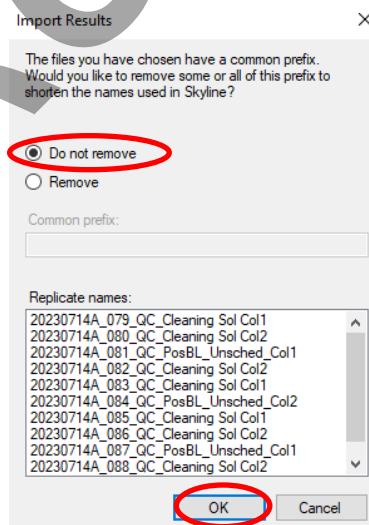| Body Fluid Identification by Proteomic Mass Spectrometry -Analysis		
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2.12   Select samples in your batch in FBiology → Molecular Serology_Data (chose year and month).

    2.12.1   If the LCMS batch has more than one case, ensure you process the controls and each case as their own individual set.

    2.12.2   If the LCMS batch only contains one case, ensure you process the controls and the case samples as the same set.

2.13   Click "Do not remove". Click OK.

2.14   Save file once all sample injections are imported and close software.

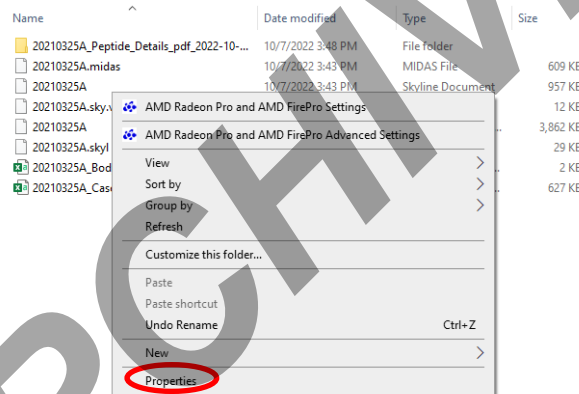# 3   MSDA (Molecular Serology Data Analysis)-Script Procedure

3.1   Find the bfassay_cmd_ps Shortcut icon on the processing computer desktop.
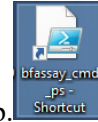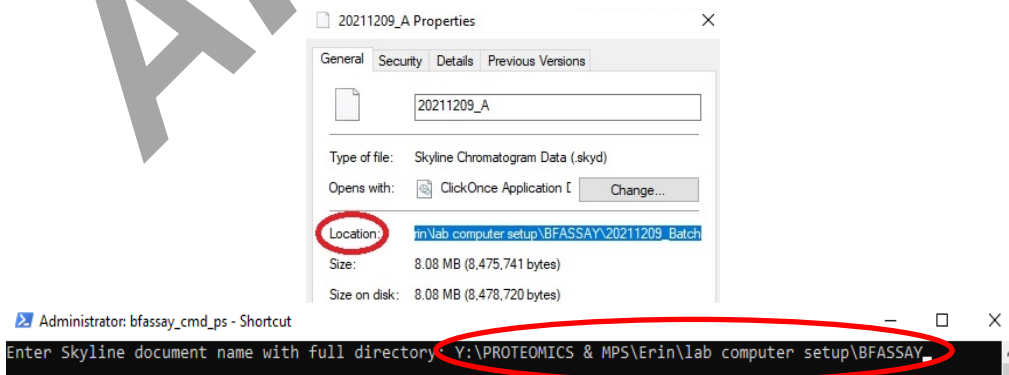
3.2   Right click on the bfassay_cmd_ps Shortcut icon and select Run with PowerShell.
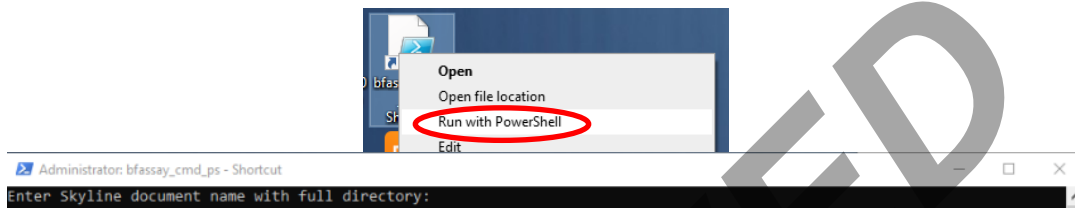
3.3   Right click on the skyline file and select Properties.

3.4   Copy the location of the file and paste into bfassay_cmd_ps command window.

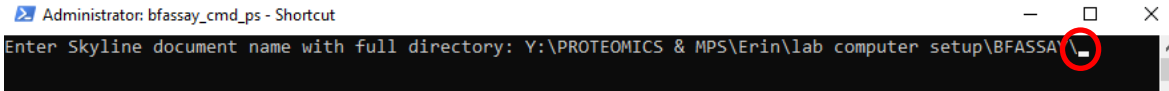3.5   Write " \ " after pasting the location.

3.6   Copy the file name and paste in into bfassay_cmd_ps command window
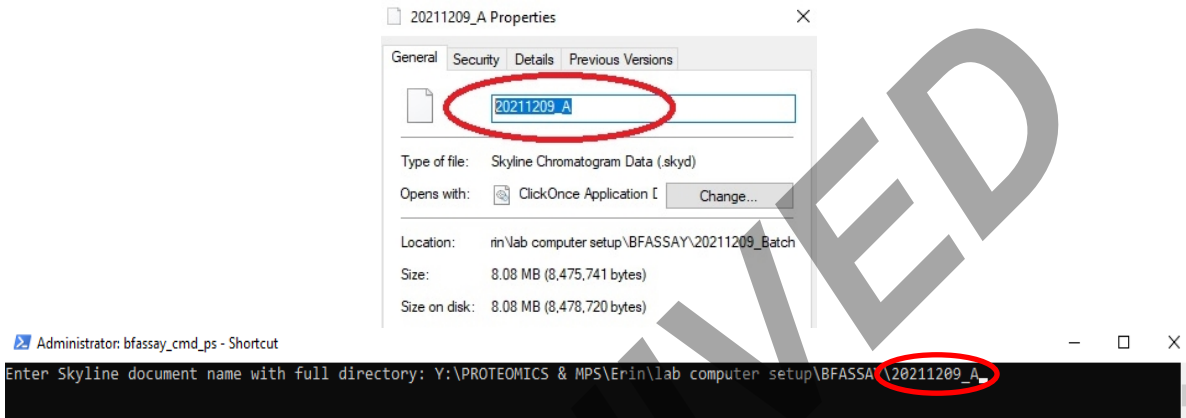
3.7   Write "**.sky**" then press enter.
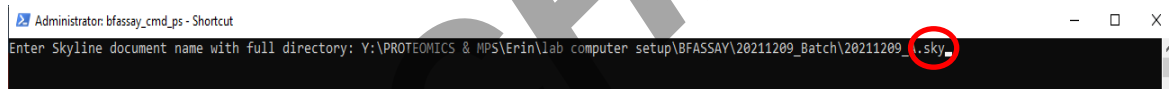
3.8   ==The script will run. The script exports will be saved in the same folder as the Skyline documents. Exports are all named starting with the same name as the Skyline document, and include a Skyline data table (Case Report), a result table for all samples (Body_Fluid_ID_Result) and a folder (Peptide_Details_pdf) containing a pdf of peptide metrics for each sample in the batch.==

3.9   ==If the interpreting analyst determines samples from the set they are analyzing need to be rerun, the analyst must perform the batch set up for the LCMS rerun in LIMS. This includes setting up the order the samples need to be run in as well as the method that needs to be used for each sample.==