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. Skyline
- 2.2 On the Start Page click on Blank Document



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

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2.6 Select File → Import File Edit Refine View Settings Start New Open	Peptide Settings Digetion Prediction filter Ubray Modifications Carbamidomethyl (C) Carbamidomethyl (C) Max variable mode: Max losses: 3 Istope label type: Istope modifications: Istope modifications: Istope label type: Istope modifications: Istope modifications:	
Open containing folder	Cth+0	
Open containing folder		
Save	Ctrl+S	
Save As		
Share		
Upload to Panorama		
Import		Results
Export		Peak Boundaries
1 M:\FBIOLOGY_MAIN\TRAINING\Prot	eomics Training\Data\NMN Skyline data\NMN IND3 data\LCMS113022 21-28.sky	Peptide Search
2 M:\PROTEOMICS MPS\lyman Almub	arak\Data 4 Reports\Case 7-JZ\Skyline\20210511A.sky	FASTA
3 M:\PROTEOMICS MPS\Iyman Almub	arak\SE 2135 Data.sky	Assay Library
4 M:\PROTEOMICS MPS\Jahaira Zapat	a\6500 buffer data\20220520_JZBabies_SDC_BeadsNoBeads_SpinCol.sky	Transition List
Exit		Document
		A 10

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

2.8 Ensure "peptides" is selected. Click OK.

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nport Transition Lis	t: Identify Column	5				
Y:\FBiology\Molecula	ar Serology_Data∖Fir	nal B SE and SA transit	tions with CytoC.csv			
Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	
Precursor m/z 🗸 🗸	Product m/z ~	r Ignore Column ∼	Peptide Modifie	Ignore Column 🗸	Ignore Columr	ı v
575.311146	937.462538	8	spIP02768 ALBU	73.1	27.2	
575.311146	823.41961	8	sp P02768 ALBU	73.1	27.2	
575.311146	694.377017	8	spIP02768IALBU	73.1	27.2	
575.311146	595.308603	8	spIP02768 ALBU	73.1	27.2	
440.724175	680.361367	8	spIP02768 ALBU	63.2	20.6	
440.724175	533.292953	8	spIP02768 ALBU	63.2	20.6	
440.724175	462.255839	8	spIP02768 ALBU	63.2	20.6	
440.724175	348.155397	8	spIP02768IALBU	63.2	20.6	
466.763634	774.450851	8	sp P68871 HBB	65.1	21.9	
466.763634	675.382437	8	sp P68871 HBB	65.1	21.9	
466.763634	574.334758	8	sp P68871 HBB	65.1	21.9	
466.763634	390.21358	8	sp P68871 HBB	65.1	21.9	
	007 450101	8	splP68871IHBB	79.1	31.2	

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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 \rightarrow Import \rightarrow Results

Skyline - Flnal B SE and SA trainsitions with CytoC and DI_2021Feb2.sky		
le Edit Refine View Settings Tools Help		
Start		
New	Ctrl+N	
Open	Ctrl+O	
Open containing folder		
Save	Ctrl+S	
Save As		
Share		
Upload to Panorama		
Import	•	Results
Export	•	Peak Boundaries
1 FInal B SE and SA trainsitions with CytoC and DI_2021Feb2.sky		Peptide Search
2 M:\PROTEOMICS MPS\lyman Almubarak\For TPP\20211216_205781Kit_IA.sky		FASTA
3 M:\PROTEOMICS MPS\lyman Almubarak\Validation 2021 BF Skyline Files\20211209_A2.sky		Accay Library
4 M:\PROTEOMICS MPS\000 Molecular BF Validation\Internal Validation 2020 Data\Final B SE and SA trainsitions with CytoC an	nd DI_2021Feb2.sky	Transition List
Fvit		Desument
		bocument
		Appotations

2.11 Click OK

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	Import Results X • Add single-injection replicates in files Optimizing: None Cancel Cancel Cancel • Add onulti-injection replicates in directories Add onulti-injection replicates in directories • Add one new replicate Name: • Add files to an existing replicate • Add files to an existing replicate Name: • Files to import simultaneously: • Files to import simultaneously: • Show chromatograms during import • Show chromatograms during import • Betry affer import failure	

2.12 Select samples in your batch in FBiology \rightarrow 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.

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

2.13	Click "Do not remove". Clicl	¢ OK.	
		Import Results	×
		The files you have chosen have a common prefix. Would you like to remove some or all of this prefix to shorten the names used in Skyline?	
		Do not remove Remove Common prefix:	
		Renlicate names:	
		Displace Producate Frames 20230714A 079. QC Cleaning Sol Col1 20230714A 080_QC Cleaning Sol Col2 20230714A 081_QC Cleaning Sol Col2 20230714A 082_QC Cleaning Sol Col2 20230714A 082_QC Cleaning Sol Col2 20230714A 083_QC Cleaning Sol Col1 20230714A 084_QC Cleaning Sol Col1 20230714A 085_QC Cleaning Sol Col2 20230714A 087_QC Cleaning Sol Col2 20230714A 087_QC Cleaning Sol Col2	•
		OK Cancel	

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

MSDA (Molecular Serology Data Analysis)-Script Procedure 3

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- 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.

	pen pen file location un with PowerShell
Administrator: bfassay_cmd_ps - Shortcut Enter Skyline document name with full directory:	
Right click on the skyline file and sel	Acct Properties.

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

	General Security Details Previous Versions		
· · ·	20211209_A		
	Type of file: Skyline Chromatogram Data (.skyd)		
	Location in Vab computer setup/BFASSAY/20211209_Batch		
	Size: 8.08 MB (8,475,741 bytes)		
	Size on disk: 8.08 MB (8,478,720 bytes)		
		_	~

3.5 Write "\" after pasting the location.

3.3

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Body Fluid Identification by Proteomic Mass Spectrometry -Analysis Status: Published Document ID: 77459 DATE EFFECTIVE APPROVED BY PAGE 6 OF 6 08/23/2023 Molecular Serology Technical Leader Administrator: bfassay_cmd_ps - Shortcut Х nter Skyline document name with full directory: Y:\PROTEOMICS & MPS\Erin\lab ^_ 3.6 Copy the file name and paste in into bfassay cmd ps command window 20211209 A Properties × General Security Details Previous Versions 20211209 A Skyline Chromatogram Data (.skyd) Type of file ClickOnce Application [Opens with: Change rin Vab computer setup \BFASSAY\20211209_Batch Location: 8.08 MB (8,475,741 bytes) Size Size on disk: 8.08 MB (8,478,720 bytes) Administrator: bfassay_cmd_ps - Shortcut X nter Skyline document name with full directory: Y:\PR OMICS & MPS\Erin\lab computer setup\BFASSA(\20211209 A_ 3.7 Write ".sky" then press enter. Administrator: bfassay_cmd_ps - Shortcut Х ter Skyline document name with OMICS & MPS\Erin\lab computer setup\BEASSAY\ .sky

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- 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.
- If the interpreting analyst determines samples from the set they are analyzing need to be rerun, 3.9 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.