Proteomics International

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IN CONFIDENCE
Client Name/Address

Purchase order Number:

5th October 2016

***De novo* sequencing analysis**
Report D161005PIJK_PI-Ref
Example Report

SAMPLES

PI-reference: 1234
Date received: 01/10/2016
Number of samples: 1
Type of samples: Human cell line
Source of sample: Gel-band/Freeze-dried sample
Service required: *De novo* sequencing analysis

Samples were labeled as:

Sample name	Molecular Mass (kDa)	PI-reference
X1	100	1234

METHODS

Protein samples were trypsin digested and peptides extracted according to standard techniques (Bringans et al. Proteomics 2008). Peptides were analysed by electrospray ionisation mass spectrometry using the Shimadzu Prominence nano HPLC system [Shimadzu] coupled to a 5600 TripleTOF mass spectrometer [AB Sciex]. Tryptic peptides were loaded onto an Agilent Zorbax 300SB-C18, 3.5 µm [Agilent Technologies] and separated with a linear gradient of water/acetonitrile/0.1% formic acid (v/v).

MS/MS spectra were analysed using PEAKS Studio Version 4.5 SP2 [Bioinformatics Solutions] and manual interpretation.

General comments for *de novo* sequencing:
* MS/MS interpretations represent a best fit of amino acids to the data; consequently sequences may not be exact.
* Ile/Leu are indistinguishable by MS and as such are interchangeable within a sequence.
* Lys (K) and Glu (Q) have near identical masses, hence an internal Q may represent an internal K cleavage site that has been missed.
* Arg/Lys can be present internally in a sequence if a trypsin enzyme cleavage site has been missed.

Accredited for compliance with ISO/IEC 17025, NATA accredited list of SOP's:
0_14 In-gel destain, reduction/alkylation, digestion & extraction of peptides
1_05 Operation of LC MS Instruments
1_09 De novo sequencing

SOPs carried out at Proteomics International Facility, located at Harry Perkins Institute of Medical Research, QEII Medical Centre, Nedlands, Perth, Australia.

RESULTS

The results provided relate only to the samples as listed above.

De novo sequencing results for 1 major peptide present in sample PI-Ref are shown in Tables 1 and 1a and the corresponding Figure 1.

In Table 1, the second and third columns show the sequential numbers and the m/z of the parent ions while the fourth column contains the deduced sequences. A maximum of 5 sequences (*i.e.* interpretations) are presented for any given ion. These are ranked based on their likelihood (0 to 4) with the ranking shown in the sixth column. The fifth column (titled Score) is also related to this ranking and shows the likelihood of any sequence amongst all possible interpretations for an ion.

Colors used to display the actual amino acids are indicative of the confidence in each amino acid assignment. The color-coded confidences are:
Red: >90% confident
Purple: 90-80% confident
Blue: 80-60% confident
Black: <60% confident

Amino acids assigned with confidence below 80% should only be used with caution. Fragment sequences for any given sample may be used individually or collectively to interrogate protein databases using a variety of algorithms. Nevertheless, the specialized algorithm for mass spectrometric analysis, MS BLAST, is often preferred over the general purpose programs such as NCBI BLAST.
The algorithm may be found at:
MS BLAST* → http://genetics.bwh.harvard.edu/msblast/

*The original article by Shevchenko *et.al* (2001) that explains the application of MS BLAST may be found on this webpage.

All results and data will be available until 2018.

Table 1: Sequencing results of sample PI-Ref

	No.	m/z	Peptide	Score (%)	Rank
PI-Ref	1	543.30	EPNKFVVPR	100	0
			EPNQFVVPR	0	1
			EPGGKFVVPR	0	2
			EPNGAFVVPR	0	3
			EPNAGFVVPR	0	4

Table 1a: Sequencing result for peptide m/z 543.30 $(MH_2)^{2+}$ (PI-Ref).
Sequence: EPNKFVVPR

#	b	sequence	y	#
1		E		9
2	212.11	P	956.56	8
3	341.14	N	859.51	7
4		K	745.47	6
5	616.29	F	617.37	5
6	715.36	V	470.31	4
7	814.44	V	371.24	3
8		P	272.17	2
9		R	175.12	1

= detected y and b ions

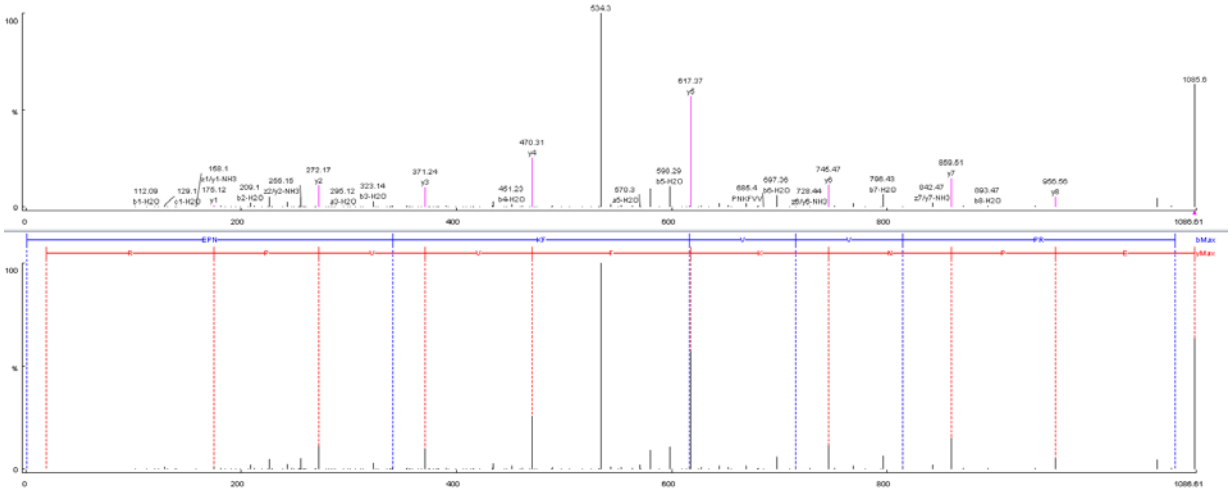

Figure 1: *De novo* spectra for peptide m/z 543.30 $(MH_2)^{2+}$ (PI-Ref).
Reference: 161003

With Compliments

Proteomics International

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