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IN CONFIDENCE
Client
Address

16th September 2013

Differential Expression by iTRAQ analysis
Report IQ130916PIAL_report template

SAMPLES

PI reference:
Date of receive:
Number of samples:
Source of sample:
Service required: Differential Expression by iTRAQ analysis

METHODS

The protein samples were acetone precipitated, reduced, alkylated and trypsin digested according to the iTRAQ protocol (Applied Biosystems). Samples were then labeled using the iTRAQ reagents as follow:

Sample name	iTRAQ reagent
1	114
2	115
3	116
4	117

Peptides were desalted on a Strata-X 33 µm polymeric reversed phase column (Phenomenex) and dissolved in a buffer containing 10mM KH2PO4 pH3 in 10% acetonitrile before separation by strong cation exchange liquid chromatography (SCX) on an Agilent 1100 HPLC system using a PolySulfoethyl column (4.6 x 100 mm, 5 µm, 300 A). Peptides were eluted with a linear gradient of 0-400 mM KCl. Eight fractions containing the peptides were collected and after desalting on Strata-X columns were loaded onto a Agilent Zorbax 300SB-C18, 3.5 µm (Agilent Technologies) running on an Shimadzu Prominence nano HPLC system [Shimadzu]. Peptides were resolved with a gradient of 10-40% acetonitrile (0.1% trifluoroacetic acid) over 160 minutes and analysed on a 5600 TripleTOF mass spectrometer [AB Sciex].

DATA ANALYSIS

Spectral data were analysed against the Swiss-Prot database using ProteinPilot™ 4.0 Software (AB Sciex) and taxonomy set to XXXX. The database was downloaded date and contained XXXXX sequences.

Accredited for compliance with ISO/IEC 17025, NATA accredited list of SOP's:

0_11 Sample Prep for iTRAQ[1]
0_12 Solid Phase Extraction[1]
8_04 Protein Pilot[2]
3_01 HPLC Agilent[2]
3_05 HPLC Cation Exchange[2]

Non NATA accredited list of SOP's

1_12 Operation of 5600 Triple-TOF Mass Spectrometer[2]

SOPs carried out at Proteomics International Facility, located at [1]Bayliss Building, UWA campus, Crawley, Australia and [2]MRF Building, 50 Murray Street, Perth, Australia.

RESULTS

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

A summary of the results is shown below.

Features	Value
No. of proteins identified	XXX
Global FDR	XXX
Local FDR	XXX
Number of MS/MS spectra used	XXX
Confidence level of protein detection	XXX
Unused ProtScore cut off	XXX
No. of differentially expressed proteins	
115 :114 117:114 116:114	XXX XXX XXX

The full report with details of the identified proteins is shown in the attached spreadsheet.

IQ130916PIAL_results (report template).xlsx

NOTES ON INTERPRETING THE RESULTS

Unused (ProtScore)
A measure of the protein confidence for a detected protein, calculated from the peptide confidence for peptides from spectra that have not already been completely used by higher scoring winning proteins. For 95% confidence, the required Unused ProtScore is 1.3.

Total (ProtScore)
A measure of the total amount of evidence for a detected protein. The Total ProtScore is calculated using all of the peptides detected for the protein. The Total ProtScore does **not** indicate the percent confidence for the identification of a protein.

% Cov (Coverage)
The percentage of matching amino acids from identified peptides divided by the total number of amino acids in the sequence.

Ratio
The average ratio for the protein, relative to 114.

The p-value
For each protein ratio reported the program calculates a p-value to help you assess whether changes in protein expression are real or not.
The p-value reports the probability that the null hypothesis (that is, the observed value is different from unity by chance) is true. P-values range from 0 to 1. A p-value of less than or equal to 0.05 indicates statistically significant differential expression and is highlighted in your results.

False discovery rate (FDR)

The FDR was automatically calculated by the Proteomics System Performance Evaluation Pipeline (PSPEP) feature in the ProteinPilot™ software using the reversed version of the protein sequences contained in the search database. The software calculates both a local and a global FDR.

The local FDR estimates the “local” error rate around a given identification, which indicates the likelihood that the specific identification is incorrect.

The global FDR estimates the error rate of the whole “global” set of answers defined by a threshold value. That is, the global FDR estimates the likely error rate of the entire set of identifications with scores as good as or better than the threshold.

With Compliments

Proteomics International

Authorised Signatory's name
Andreja Livk
Contract Services Manager

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