

Quantitative Proteomics- iTRAQ Labeling

Client Name

Service Number #123 (12pt)

XXXX/XX/XX(12pt)

Quantitative Proteomics- iTRAQ Labeling

Analysis Information

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I. Sample name:

1. #1
2. #2
3. #3
4. #4

II. Sample information:

1. Received date: 2021/01/01
2. Received status: Frozen
3. Sample type: Protein solution

III. Analysis Method:

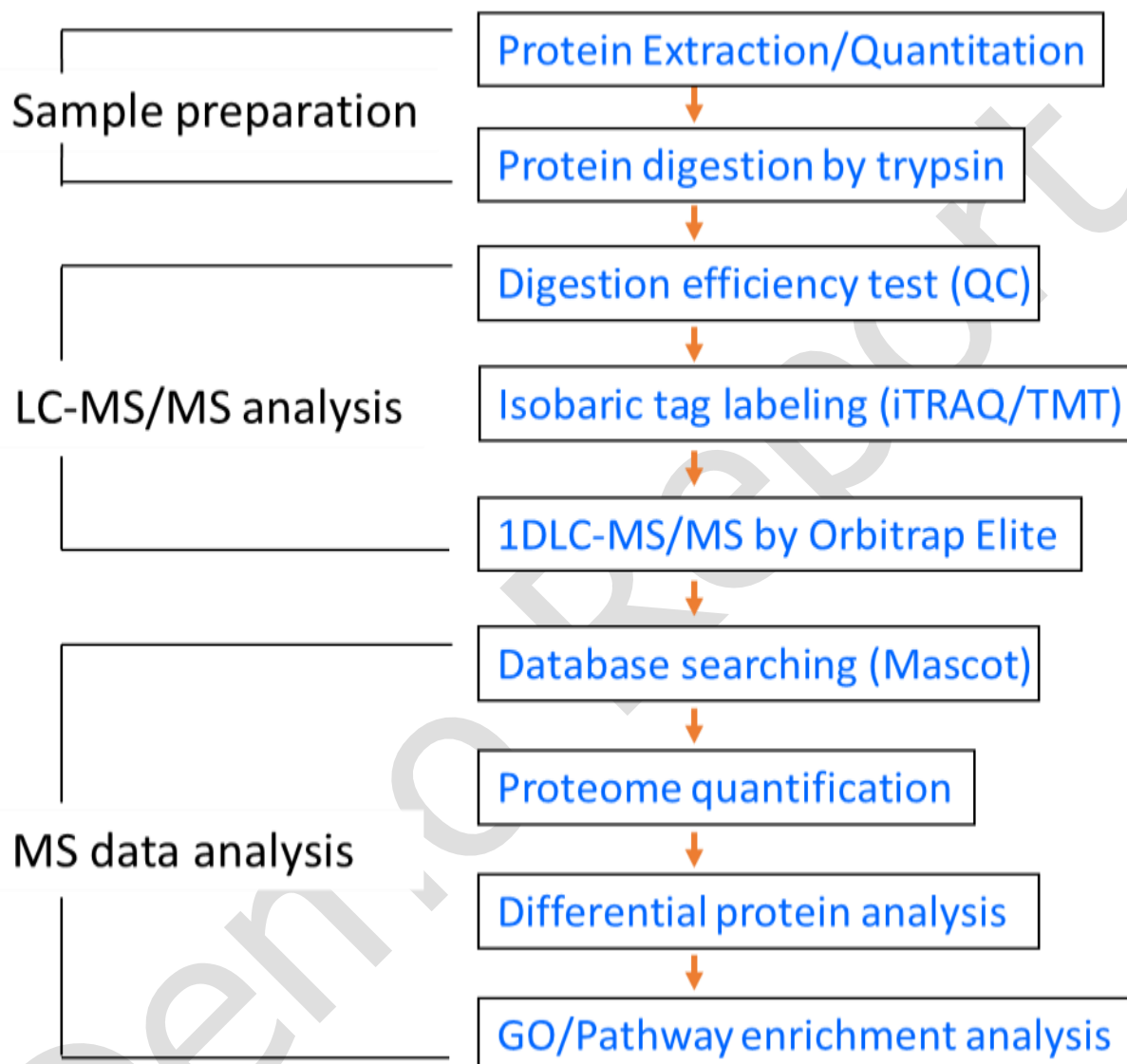
1. In-solution digestion with iTRAQ reagent labeling
2. Liquid chromatography based tandem mass spectrometry (LC-MS/MS)
3. Peptide fragmentation pattern - database search (algorithm: Mascot v.2.5)
4. Peptide validation - false discovery rate (FDR algorithm: Percolator)

Analysis Workflow

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Analysis Results

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1. Summary of protein identification

The proteins in solution were successfully identified as below (see appendix files for further detail).

Sample (QC)	Protein ID	Peptide ID	PSM	Search input
#1	733	3681	10539	16944
#2	838	4790	12264	18556
#3	621	2870	9040	16124
#4	920	5571	12799	18960
Sample	Protein ID	Peptide ID	PSM	Search input
iTRAQ4plex	824	5842	16255	27931

2. Data reduction filters:

- Peptide Confidence: High ($FDR \leq 0.01$)
- Peptide Length: 7-100 amino acids
- Peptide Rank: 1
- Minimal Peptides Per Protein: 2 (count only rank 1 peptides in top scored proteins)

3. Search parameters:

- Protein Database: Swissprot database
- Enzyme Name: Trypsin
- Maximum Missed Cleavage Sites: 2
- Taxonomy: *Mus musculus*
- Precursor Mass Tolerance: 10 ppm
- Fragment Mass Tolerance: 0.2 Da (HCD)/0.5 Da (CID)
- Dynamic Modification: Oxidation (M), Acetyl (Protein N-term), and iTRAQ4plex (peptide N-terminal, K)
- Static Modification: Methylthio (C)

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4. Summary of quantification results

# Proteins	Total Quantification	$\geq \text{mean} + 1.5 \times \text{s.d.}$	$\leq \text{mean} - 1.5 \times \text{s.d.}$
#2/#1	798	26	40
#3/#1	798	36	38
#4/#1	797	29	43

5. Summary of gene ontology (GO) and pathway enrichment

Trend	Category	#2/#1	#3/#1	#4/#1
Enrichment analysis by	GO	23	28	42
up -regulated protein	Pathway	4	13	3
Enrichment analysis by	GO	24	41	29
down -regulated protein	Pathway	3	3	3

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In-sol digestion. The protein solutions were first diluted in 200 mM triethylammonium bicarbonate (TEABC), and then reduced with 5 mM tris-(2-carboxyethyl)-phosphine (TCEP, Sigma-Aldrich) at 60°C for 45 min, followed by cysteine-blocking with 10 mM methyl methanethiosulfonate (MMTS, Sigma-Aldrich) at 25°C for 30 min. Samples were digested with sequencing-grade modified porcine trypsin (Promega) at 37°C for 16 hours. The peptides were labeled with iTRAQ reagent for 1 hour at room temperature, pooled and dried by vacuum centrifugation. Sample was store at -20 °C until use.

LC-MS/MS analysis. The dried peptide mixtures were reconstituted in HPLC buffer A (0.1% formic acid) and loaded onto a reverse-phase column (Zorbax 300SB-C18, 0.3 ´ 5 mm; Agilent Technologies). The desalted peptides were then separated on C18 column (Waters BEH 1.7 µm, 100 µm I.D. × 10 cm) using a multi-step gradient of HPLC buffer B (99.9% acetonitrile/0.1% formic acid) for 120 minutes with a flow rate of 0.3 µl/min. The LC apparatus was coupled with Orbitrap Elite MS (Thermo Fisher Scientific) operated using Xcalibur 2.2. The full-scan MS was performed in the Orbitrap over a range of 400 to 2,000 Da with resolution of 120,000 at m/z 400. The 16 data-dependent MS/MS scan events (8 CID, 8 HCD) were followed by one MS scan for the 8 most abundant precursor ions in the preview MS scan. The m/z values selected for MS/MS were dynamically excluded for 80 seconds with a relative mass window of 15 ppm. The electrospray voltage was set to 2.0 kV, and the temperature of the capillary was set to 200°C. MS and MS/MS automatic gain control were set to 1,000 ms (full scan) and 200/300 ms (CID/HCD), or 3 ´ 10⁶ ions (full scan) and 3 ´ 10³/3 ´ 10⁴ ions (CID/HCD) for maximum accumulated time or ions, respectively.

Protein identification and quantification. The data analysis was carried out using Proteome Discoverer software (version 2.3, Thermo Fisher Scientific), including the reporter ions quantifier node for iTRAQ

quantification. The MS/MS spectra were searched against the Swissprot database using the Mascot search engine (Matrix Science, version 2.5). For peptide identification, 10 ppm mass tolerance was permitted for intact peptide masses, and 0.2 Da for HCD/0.5 Da for CID fragment ions with allowance for two missed cleavages made from the trypsin digestion: oxidation (M), acetyl (protein N-terminal), and iTRAQ4plex (peptide N- terminal, K) as variable modifications; methylthio (C) as static modification. Peptide-spectrum match (PSM) were filtered based on high confidence and search engine rank 1 of peptide identification to ensure an overall false discovery rate below 0.01. Proteins with single peptide hit were removed. The quantitative data of protein were exported from Proteome Discoverer using integration methods of most confident centroid with 20 ppm tolerance. The protein ratios in each group were normalized using median ratio of each group. The differential proteins were then determined using threshold: mean of log₂ transformed ratio ± 1.5 × standard deviation of log₂ transformed ratio, assuming normalized ratio distributed normally. The Gene ontology and Pathway of differential proteins were then enriched using DAVID database (<https://david.ncifcrf.gov>).

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1. Protein-peptide QC Report

Accession	Description	MW [kDa]	calc. pI	Score	Coverage	# AAs	# Proteins	# Unique Peptides	# Peptides	# PSMs	Area	Relative abundance (%)
P63260	Actin, cytoplasmic 2 OS=Mus musculus C57BL/6J	41.8	5.48	4113.29	75.20	375	1	10	22	144	3.282E9	1.1%
	Sequence	Protein Group Accession	Area	# PSMs	q-Value	Ion Score	m/z [Da]	Charge	RT [min]	Modifications	# Missed Cleavages	
	GVSTTTAER	P63260	2.072E9	7	0	73	566.78715	2	22.49		0	
	QEDDSQPSIVRR	P63260	8.517E8	4	0	62	758.85596	2	20.34		0	
	NPDAALVINGSGMRK	P63260	1.426E7	2	0	139	933.91862	2	60.30	N-Term(Acetyl); C16	0	
	YVEHGIVTNWDMRK	P63260	1.246E7	1	0	27	649.63892	3	36.82		0	
	VAPETHPVLTTPANPK	P63260	4.368E8	1	0	73	677.53644	2	22.09		0	
	VEEETAAVINGSGMRK	P63260	1.055E9	1	0	38	955.43884	2	64.25	N-Term(Acetyl); C16	0	
	DLVANTVSGGTTMYPGADR	P63260	2.891E8	11	0	87	1108.03955	2	42.48		0	
	NOLYANTVLSGGTTMYPGADR	P63260	4.045E8	4	0	45	761.72711	3	37.89		1	
	LPTVALQPEQMATAASSSSLRK	P63260	4.624E8	14	0	144	1270.07385	2	59.34	C2(Methylthio); C16	0	
	QPALQPSFLQESGDHETTPNSMRK	P63260	7.962E7	1	0	44	1070.47046	3	56.36	C1(Methylthio); C16	0	

Column title descriptions:

Protein level	
Column name	Description
Accession	Master Protein 資料庫代碼
Description	Master Protein 的名稱與功能描述
MW [kDa]	Master Protein 依據 sequence database 預測的分子量
calc. pI	Master Protein 依據 sequence database 預測的等電點
Score	MudPit score; 該蛋白(Master Protein) 的鑑定到所有 Spectrum ion score 的總和。
Coverage	鑑定到的 Peptide 序列佔完整 Master Protein 序列的比例
#AAs	Master Protein 依據 sequence database 計算的氨基酸數目
#Proteins	與 Master Protein 歸屬於同一個 Protein Group 的 Protein number (以該 protein group 裡的 protein 的 #PSMs、Scores 和 Coverage 最高的 Protein 當作這個 group 的 Master Protein)
#Unique peptides	鑑定到的 Peptide 序列只專屬於該蛋白(Master Protein) 的 Peptide 總數目
#Peptides	鑑定到的 Peptide 序列屬於該蛋白(Master Protein) 以及跟其他 Protein group 的 sequence 相同的 Peptide 數目 (shared peptide)
#PSMs	鑑定到的 Spectrum 屬於該蛋白(Master Protein) 的 Spectrum 總數目
Area	該蛋白(Master Protein) 鑑定到的 Unique peptide 中, abundance 最高的 3 個 Unique Peptide Area 的平均值。
Relative Protein Abundance [%]	Master Protein 在樣本中的相對豐富度(不包括被判斷為污染的蛋白質, e.g. Keratin 等), 計算公式: #PSM / (#Total PSM) * 100
Peptide level	
Column name	Description
Sequence	比對到的 Peptide 的序列
Area	Peptide 在 LC/MS 分析中被偵測到的面積
#PSMs	被鑑定到屬於該段 Peptide 的 spectrum 數目
q-value	Peptide 鑑定結果之錯誤發生率 (False discovery rate)
Ion Score	Peptide 在資料庫比對後的分數(Mascot), 越高越好。
m/z [Da]	Peptide 在 LC/MS 分析中被偵測到的 m/z (mass to charge ratio)
Charge	Peptide 在 LC/MS 分析時帶的電荷數目
RT [min]	Peptide 在 LC-MS 分析時, 該 Peptide 被質譜儀偵測到的時間 (Retention time)
#Missed Cleavages	Peptide 序列中, 應被酵素切斷, 但未被切斷的數目
Modifications	Peptide 裡可能被修飾的種類以及可能被修飾 amino acid 位點

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2. Protein-peptide Report

Accession	Description	Abundance Ratio #2 / #1	Abundance Ratio #3 / #1	Abundance Ratio #4 / #1	Sum PEP Score	Coverage [%]	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	pI
Q52881	Human H4 (C14-Myc mouse) [C14-Myc]	9.737	3.262	0.138	287.263	86	46	13	13	1	80	11.4	10.9
Master Peptide Accessions Master Peptide Descriptions Annotated Sequence # PSMs Modifications # Missed Cleavages Theo. MH+ [Da] m/z [Da] DeltaM [ppm] (by Search Engine) (by Search Engine) (by Search Engine) (by Search Engine) # Proteins # Protein Groups													
P12808	Human H4 (C14-Myc mouse) [C14-Myc]	471154211142.35			277942220.35	100%	1	1	1	1	80	11.4	10.9
P12808	Human H4 (C14-Myc mouse) [C14-Myc]	471154211142.35			277942220.35	100%	1	1	1	1	80	11.4	10.9
P12808	Human H4 (C14-Myc mouse) [C14-Myc]	471154211142.35			277942220.35	100%	1	1	1	1	80	11.4	10.9
P12808	Human H4 (C14-Myc mouse) [C14-Myc]	471154211142.35			277942220.35	100%	1	1	1	1	80	11.4	10.9

Column title descriptions:

Protein level	
Column title	Description
Accession	Master protein 資料庫代碼
Description	Master protein 名稱與功能描述
Abundance Ratio	Tag intensity 為基準去計算各組的 protein ratio
Abundance Ratio Variability [%]	用於計算ratio之數值間的變異數
Sum PEP Score	PEP Score (posterior error probability) : peptide 跟 PSM 鑑定錯誤率 Sum PEP Score則為PEP總和
Coverage [%]	鑑定到的peptide占完整master protein序列之比例
# Peptides	鑑定到的peptide序列只專屬於該Master protein的peptide 及跟其他protein group相同的peptide數目 (shared peptide)
# PSMs	鑑定到的spectrum專屬於該Master protein的spectrum總數
# Unique Peptides	鑑定到的peptide序列只專屬於該Master protein的peptide總數
# Protein Groups	與master protein歸屬於同一個Protein Group的protein number
# AAs	Master protein 依據sequence database計算得胺基酸數目
MW [kDa]	Master protein 依據sequence database預測之分子量
pI	Master protein 依據sequence database預測之等電點
Score Mascot: Mascot	該protein 鑑定到之spectrum ion score之總和
Abundance	peak intensity計算PSM abundance後，再加組成peptide abundance及protein abundance
Abundances Count	用於計算protein abundance的peptide group數目
Found in Sample	用於判斷該Master protein鑑定到的PSM之可信度 high:high confidence Peak found:found but unidentified PSM Not found:NO PSM detected in the sample
Peptide level	
Column title	Description
Master protein Accession	Master protein 資料庫代碼
Master protein Description	Master protein 名稱與功能描述
Annotated Sequence	比對到的peptide序列
# PSMs	鑑定到的spectrum專屬於該peptide的spectrum總數
Modifications	Modification在該peptide上的位點
# Missed Cleavages	Peptide序列中應被酵素切斷卻沒被切斷的位點
Theo. MH+ [Da]	Peptide理論質量數
m/z [Da] (by Search Engine): Mascot	Peptide在LC/MS分析時被偵測到的m/z (mass to charge ratio)
DeltaM [ppm] (by Search Engine): Mascot	Peptide在LC/MS分析時被偵測到的m/z與該peptide m/z計算值的差異
Ions Score (by Search Engine): Mascot	Peptide在資料庫比對的分數
Quality q-value	Peptide 鑑定結果之錯誤發生率 (False Discovery Rate)
# Proteins	序列中包含該peptide的protein number
Abundance Ratio	比較組別之Peptide abundance ratio值
Abundance	peak intensity計算PSM abundance後，再加組成peptide abundance
Abundances Count	用於計算peptide abundance的PSM 數目

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3. Differential Protein Report

Accession	Description	Gene name	Normalized ratio	#(1) / #2	Trend	Variability [%]	Sum PEP Score	Coverage [%]	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Mascot	Abundance	Abundance Count	Found in Sample
P00001	Protein 1 (100-100-100-100)	P00001	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100
P00002	Protein 2 (100-100-100-100)	P00002	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100
P00003	Protein 3 (100-100-100-100)	P00003	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100
P00004	Protein 4 (100-100-100-100)	P00004	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100
P00005	Protein 5 (100-100-100-100)	P00005	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100
P00006	Protein 6 (100-100-100-100)	P00006	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100
P00007	Protein 7 (100-100-100-100)	P00007	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100
P00008	Protein 8 (100-100-100-100)	P00008	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100
P00009	Protein 9 (100-100-100-100)	P00009	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100
P00010	Protein 10 (100-100-100-100)	P00010	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100	1.100

Column title descriptions:

Protein level	
Column title	Description
Accession	Master protein 資料庫代碼
Description	Master protein 名稱與功能描述
Normalized Ratio	此蛋白質的相對表現量 (以該組所有蛋白的Median ratio進行global normalization)
Trend	此蛋白質是否為差異表現蛋白質 (以該組所有蛋白之Log2 (ratio) average+1.5*Log2 (ratio) standard deviation作為標準)
Variability [%]	用於計算ratio之數值間的變異數
Sum PEP Score	PEP Score (posterior error probability) : peptide 跟 PSM 鑑定錯誤率 Sum PEP Score則為PEP總和
Coverage [%]	鑑定到的peptide占完整master protein序列之比例
# Peptides	鑑定到的peptide序列只專屬於該Master protein的peptide 及跟其他protein group相同的peptide數目 (shared peptide)
# PSMs	鑑定到的spectrum專屬於該Master protein的spectrum總數
# Unique Peptides	鑑定到的peptide序列只專屬於該Master protein的peptide總數
# Protein Groups	與master protein歸屬於同一個Protein Group的protein number
# AAs	Master protein 依據sequence database計算得胺基酸數目
MW [kDa]	Master protein 依據sequence database預測之分子量
calc. pI	Master protein 依據sequence database預測之等電點
Score Mascot: Mascot	該protein 鑑定到之spectrum ion score之總和
Abundance	peak intensity計算PSM abundance後，再加總成peptide abundance及protein abundance
Abundances Count	用於計算protein abundance的peptide group數目
Found in Sample	用於判斷該Master protein鑑定到的PSM之可信度 high:high confidence Peak found:found but unidentified PSM Not found:NO PSM detected in the sample

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4. Gene Ontology and Pathway Enrichment Report

Trend	Category	Term	Count	Genes	%	PValue	Benjamini	FDR	Fold Enrichment
up-regulation	GOTERM_CC_DIRECT	GO:0070062~extracellular exosome	26	PRPS2, CSE1	68.42	6.93E-14	8.39E-12	7.21E-12	5.03E+00
up-regulation	GOTERM_BP_DIRECT	GO:0006457~protein folding	7	HSPA8, HSP6	18.42	1.53E-07	4.19E-05	4.19E-05	2.75E+01
up-regulation	GOTERM_CC_DIRECT	GO:0005829~cytosol	16	HSPA8, HSP6	42.11	2.99E-07	1.81E-05	1.55E-05	4.64E+00
up-regulation	GOTERM_MF_DIRECT	GO:0051082~unfolded protein bindir	6	HSPA8, HSP6	15.79	4.36E-07	5.88E-05	5.75E-05	3.77E+01
up-regulation	GOTERM_BP_DIRECT	GO:0042026~protein refolding	4	HSPA8, HSP6	10.53	5.54E-07	7.56E-05	7.56E-05	2.23E+02
up-regulation	GOTERM_BP_DIRECT	GO:0051085~chaperone mediated pr	4	HSPA8, HSP6	10.53	1.87E-06	1.71E-04	1.71E-04	1.55E+02
up-regulation	GOTERM_BP_DIRECT	GO:0006986~response to unfolded p	5	HSP90AA1, H	13.16	2.76E-06	1.88E-04	1.88E-04	4.92E+01
down-regulation	GOTERM_BP_DIRECT	GO:0006888~ER to Golgi vesicle-mex	3	SEC13, SEC2	7.50	1.23E-02	9.26E-01	9.26E-01	1.74E+01
down-regulation	GOTERM_CC_DIRECT	GO:0005915~zonula adherens	2	CTNND1, CT	5.00	1.38E-02	1.30E-01	1.18E-01	1.40E+02
down-regulation	GOTERM_CC_DIRECT	GO:0016363~nuclear matrix	3	ANP32A, CA	7.50	1.59E-02	1.40E-01	1.27E-01	1.52E+01
down-regulation	GOTERM_BP_DIRECT	GO:0014075~response to amine	2	CAD, CDK1	5.00	1.92E-02	1.00E+00	1.00E+00	1.00E+02

Column title descriptions:

Column name	Description
Trend	該 pathway 是由變化量增加或減少的差異蛋白做 enrichment analysis 得來的結果
Category	Pathway Database 名稱
Term	Enriched pathway 的資料庫代碼及名稱
Count	差異蛋白列表中, 有參與此 pathway 的蛋白質數目
Gene/Accession	差異蛋白列表中, 有參與此 pathway 的蛋白質基因名稱或蛋白質資料庫之代碼
P value	Pathway enrichment 分析產生之 P value
Benjamini	Pathway enrichment 分析產生之 P value 利用 Benjamini correction 後之 adjusted p value
FDR	Enriched pathway 的 false discovery rate
Fold Enrichment	此數值代表此 pathway enrichment 的結果, 相較於此 pathway 隨機被 enrich 出現的倍率 (fold), 倍率越高代表此 pathway 越不可能是隨機被 enrichment

