

Expression Proteomics

(paikyk@yonsei.ac.kr),

(HUPO)

I. ' - omics ' 가

가. /

(proteome) (genome) 가 (2).
 <PROTEin expressed by a
 genOME> ' () ' (isoform)⁽³⁾(2),
 1995 Siena 2 - PTM,
 Dimensional Electrophoresis(2DE) meeting
 Marc Wilkins (DNA chip)

(1). (proteomics)
 (proteome) ~ , ~
 - ics가

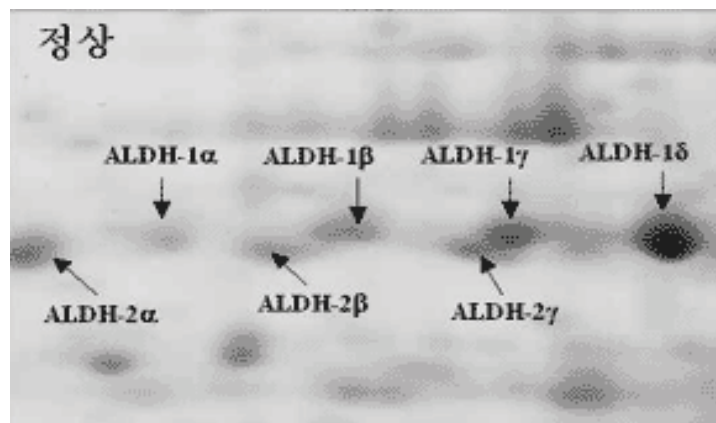
' - omics ' Proteomics Expression

가 ,

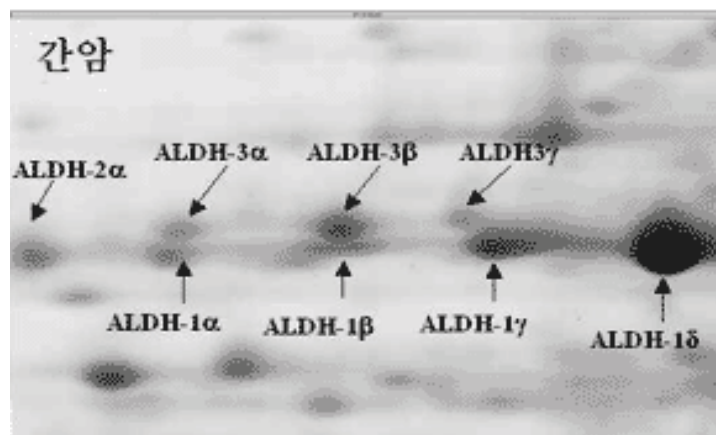
(metabolomics),
 (transcriptomics), (physiomics),
 (cheminomics),
 (pharmacogenomics), (cellomics),
 (toxicomics), (lipidomics),
 (clinomics)

' (1). ,
 (expression),
 (function), (structure)
 (post - translational modification: PTM),

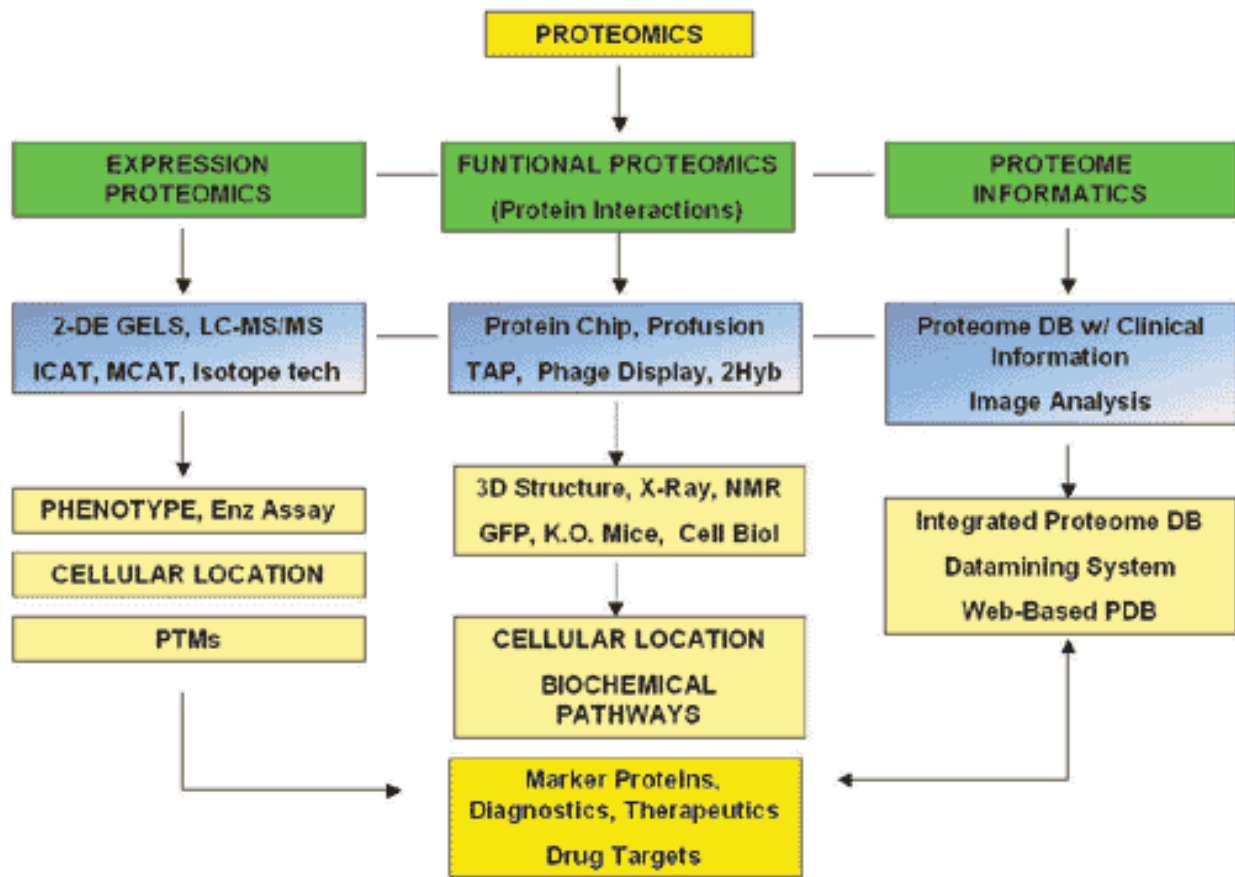
(protein - protein
interaction)



가



2 ALDH2 ALDH3
(- Ref. Park et al., Intl J. Cancer,
97, 261 - 265, 2002)



1

1 2 3 4 5 6

()

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Functional Proteomics

II. 가. 6 (Human Genome Project: HGP) HGP Proteome Project: HPP) HGP HPP 가 (3). 가 HPP (www.hupo.org) Web 가 HUPO AOHUPO() 가 가 (www.most.go.kr)가 2002 (1) (,)

genomics , structural genomics 3 가 , structural proteomics 3 HUPO (Proteome Informatics/Knowledge Base) 가

, HGP (2) (perturbation)(,
 , HPP , shock) (profiling)
 가
 Big Science (3) -
 (4) (pathway)
 mRNA level (5) folding 3
 (, (structural proteomics)
 0.5)(4), (6) (proteome
 PTM 가 informatics) DB
 (7) in vivo - -
 가 (, TATA - box).

Race on the Human Proteome Project - Another Long Journey ! www.hupo.org



AOHUPO
KHUPO

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III. Expression Proteomics (Proteome Informatics)

(1). 가

1. 2DE

가.

1975 O'Farrell 27
2 - DE

(5). 1 (pI)
2

sample) (crude
(isoform, PTM)
gel 700 1000
가

2DE IPG - DALT (:
www.apbiotech.com)
pH isoelectric
focusing strip pre - made gel

pH range strip(Zoom - in gels: , pH 3.0 - 1

5) spot batch 가

6) , high throughput :
(, OGS, Large Scale Biology)

2DE robotics

. 2DE

가 2DE

1)
(, ,)
(
,) , salt
(6).
가 ,

(,
IgG Albumin)
, affinity resin filter

2DE

4.0, 3.5 - 4.5, 4.0 - 5.0)
가

MALDI
가
silver 가

CYPRO™
가

detector

MALDI

spot
Coomassie
gel

2D - Fluorescence difference gel
electrophoresis(2D - DIGE) 2DE

(, Cy™, Dye:
Cy2, Cy3, Cy5)
DeCyder Image

MPD(Multiple Photon
Detection)

. 2DE

1) :
가
detergent
가

2) : gel load

3) :
IPG strip(pH
6 - 12) buffer

4) :
GradPore™

cysteinyI peptide capture
enrichment
(,
anhydrotrypsin)
LC - MS/MS

LCD(laser capture microdissection)

(NCI)
(7). 2DE

MCE(multi - compartment
electrolyser)(4) 가
gel loading 가
' Proteomics toolbox '

detergent cocktail high
throughput IPG strip
, alkaline pI (>pH 10)
(> 500 kDa)
Gradipore™

2) :
buffer detergent IPG strip,
batch

5) :
, buffer, IPG strip batch, gel size

3) ,
9.5 - 10.5)

pI IPG strip(, pI
buffer

1 2 3 4 5 6

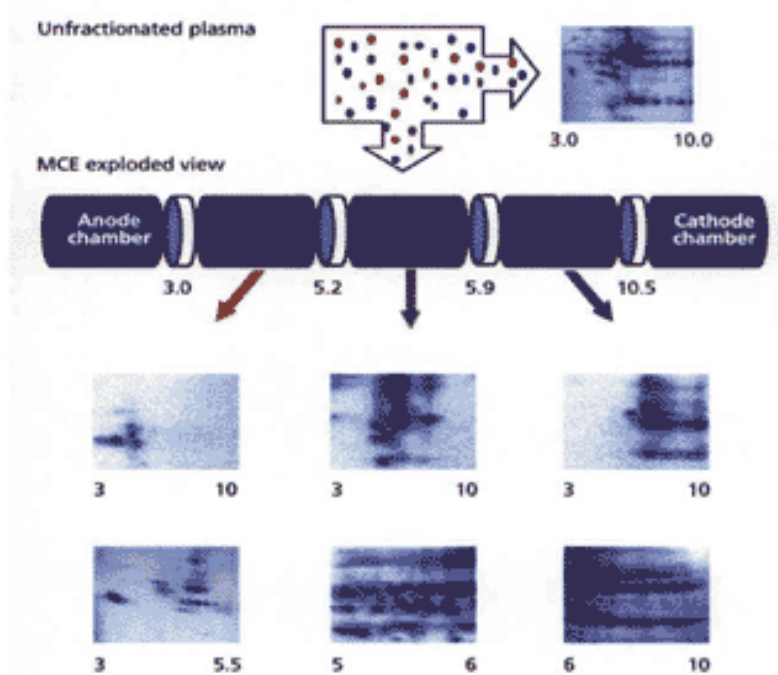
()

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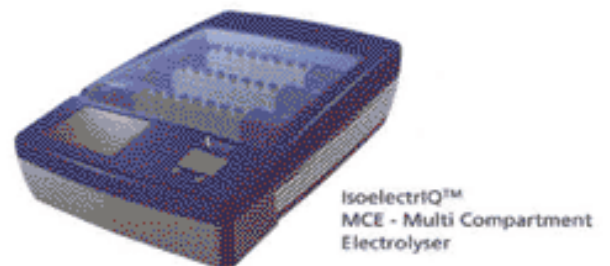
Expression Proteomics

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(HUPO)



- 단백질 샘플의 사전분획가능
- abundant proteins 의 사전 제거가능
- 2DE 젤상에서 보다 많은양의 분석가능
좁은 pH에서 단백질 스팟 분석능 향상



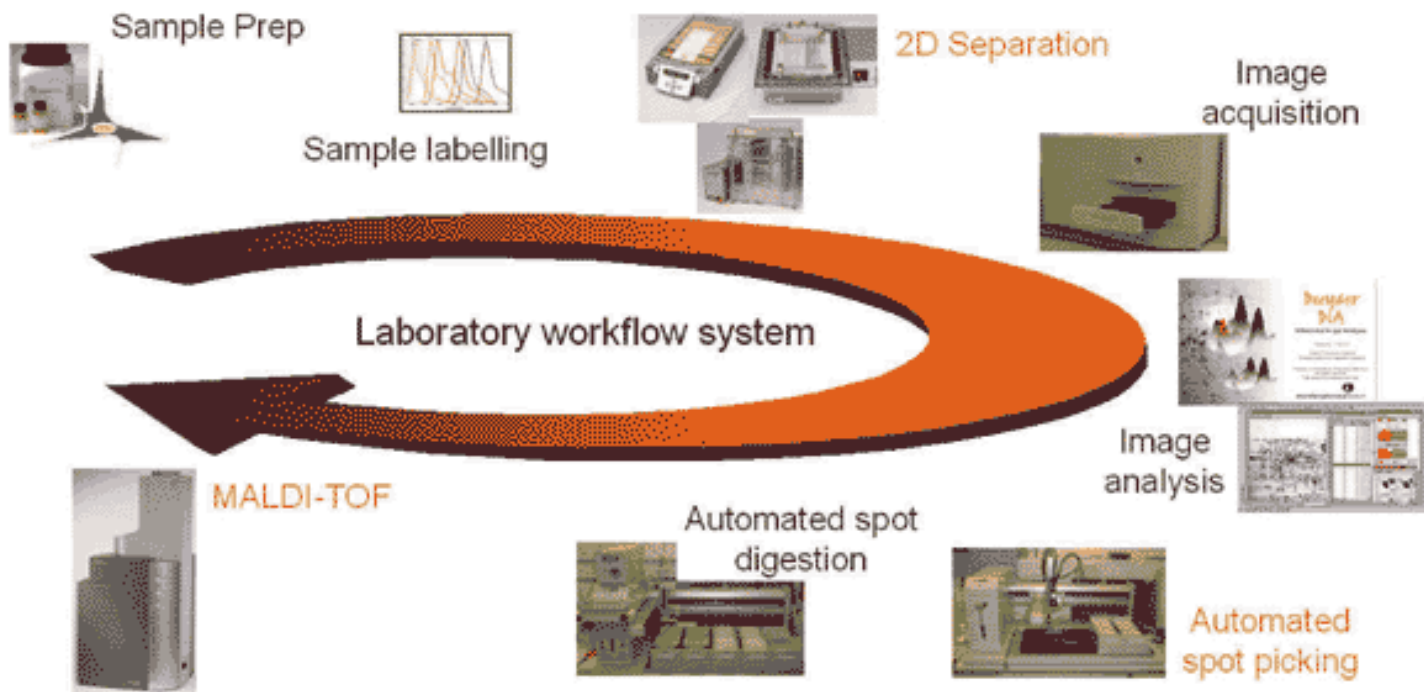
4 Multi - Compartment Electrolyser

(MCE)

4)

PCR
DNA
가
가
bottleneck
2DE
MALDI
annotation
5). 2DE
robotics
Molecular scanner
SIB(Swiss Institute of
Bioinformatics)
가 , 2DE gel
trypsin ,
PVDF membrane blotting spot
MALDI
annotation
trypsin
, blotting , spot
MALDI . ABI
(www.appliedbiosystems.com)가
Proteomics
Solution 1

MASS DB 가
workstation
submicroliter 가
, robotic arm data
S/W가
MALDI Bio - Rad
(www.biorad.com) Micromass
(www.micromass.com)가
ProteomeWorks™ spot
, MASS Data
kit가
microplate loading 가
Micromass'
MS
MS
Peptide mass fingerprinting DB
Electrospray MS/MS 가 de novo
sequencing
Genomic Solutions(www.
genomicsolutions.com)
The Investigator™ Proteomic System
2 - DE, , spot ,
ProGest(96 digest
) ProMS MALDI
가



5 2DE Expression Proteomics

1 2 3 4 5 6 ()

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Expression Proteomics

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2. 2DE Image

spot S/W가
, Melanie (GenBio), PDQuest(BioRad);
ImageMaster(Amersham Bioscience), Gellab
(Scanalytics) and Kepler (Large Scale
Proteomics), Z3(Compugen), Progenesis
(Nonlinear Dynamics)가
progenesis가

가
(,) , ,
BioBud
가 Progenesis
(Nonlinear Dynamics, UK)가
highthroughput

3. Mass Spectrometry

MALDI - MS(Matrix assisted laser desorption
ionization mass spectrophotometry)

가
(8 - 10)
microsystem(flow -
through piezo dispenser)
peptide . FTICR
MS ESI MALDI ionization 100

(Celera Genomics, OGS, LSB,
GeneProt)
(LC -
MS/MS or 2D chromatography MS/MS)
MDLC (Muti -
dimensional LC) 가 ICAT

Differential Isotope labeling

2 (e.
g. diseased vs. normal)
2DE 가
15N
2DE
peptide MASS
peptide MASS
(11).

ICAT (isotope - coded affinity tags)

ICAT cysteine 2
가 biotin
avidin resin LC ,
MASS 가
(12).
cystein
cystein 15%
가
software가
high throughput

kDa, ESI - FTICR, affinity proteomics, MALDI, MCAT (mass - coded abundance tagging), QIT (quadrupole ion trap) reflection time - of - flight (TOF) MS

MALDI가

laser

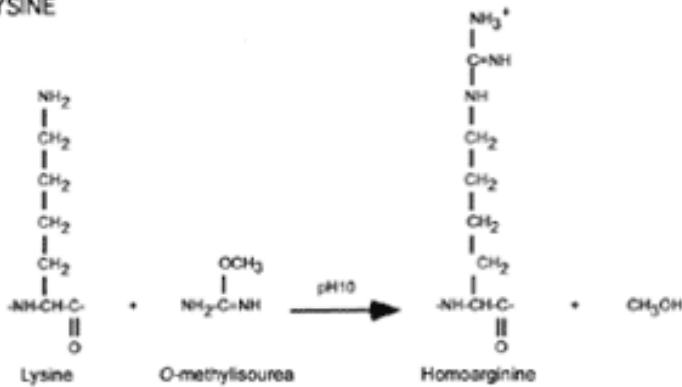
ICAT
lysine (mass - coded abundance tagging) (13) (6).
guanidination homoarginine
lysine MCAT
tryptic O - methylisourea
MCAT LC - MS
residue lysine
specific

4. ' Expression Proteomics ' Non 2DE Techniques

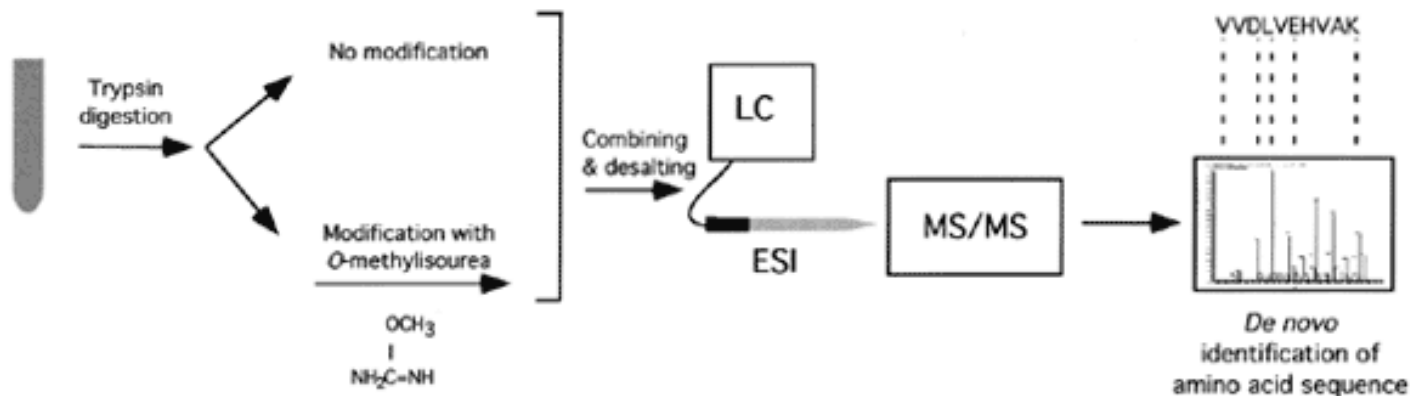
가. Non 2DE Tech

high throughput non - 2DE 2DE

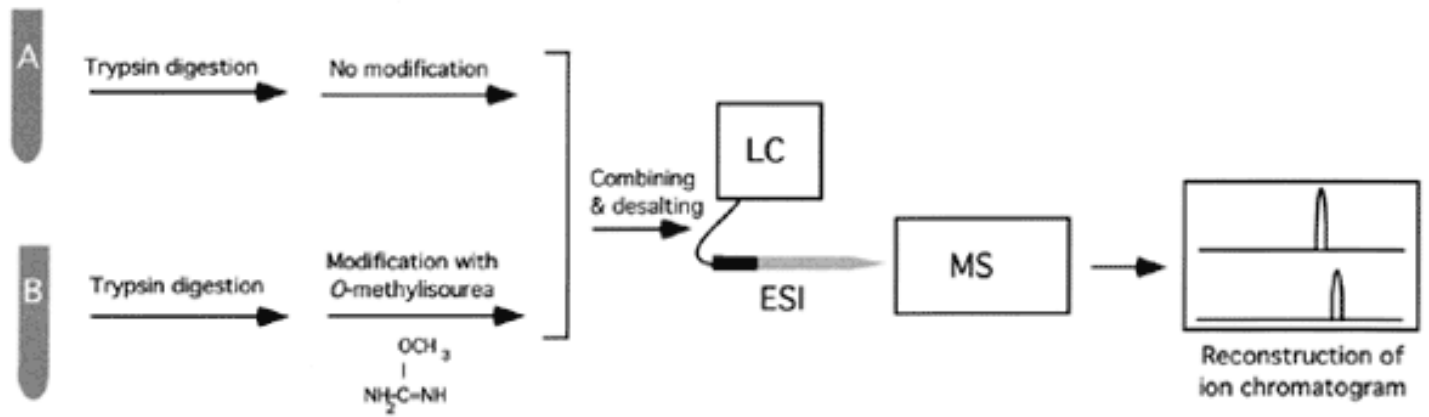
A GUANIDINATION OF LYSINE



PEPTIDE SEQUENCING



PEPTIDE QUANTITATION



6 Mass - Coded Abundance Tagging (MCAT)

1 2 3 4 5 6 ()

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(HUPO)

6. DB proteome informatics 가 , (7). 2D - DB Make2ddb gel image manage . SIB 가 2DE DB가 , query가 , tool Bio - Rad MicroMass가 WorksBase System™ history, 가 가(>USD200,000) PRIME, GlycoDB SIB HPI(Human Proteomics Initiative), HAMAP(High quality automated microbial annotation of proteomes) DB Incyte Proteome 가 DB(www.proteome.com)가 (, yeast, Candida albicans, Caenorhabditis elegans). DB (, ,) ,
4. Gygi SP, Rochon Y, Franza BR, Aebersold R. Correlation between protein and mRNA abundance in yeast. *Mol Cell Biol.* 1999 Mar;19(3):1720 - 30.
5. Link, A.J. 1999, 2D Proteome Analysis Protocol in *Methods in Mol. Biol.*, vol., 112, Humana Press, Totowa, New Jersey.
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7. Herrmann PC, Liotta LA, Petricoin EF 3rd. Cancer proteomics: the state of the art. *Dis Markers.* 2001;17(2):49 - 57. Review.
8. Mann, M., Hendrickson, R.C., Pandey, D., 2001, Analysis of protein and proteome by mass spectrometry, *Annu Rev Bioche*, 70, 437 - 73
9. Aebersold R, Goodlett DR. Mass spectrometry in proteomics. *Chem Rev.* 2001 Feb;101(2):269 - 95. Review. No abstract available.
10. Conrads TP, Issaq HJ, Veenstra TD. 2002 New tools for quantitative phosphoproteome analysis. *Biochem Biophys Res Commun.* ;290(3):885 - 90.
11. Mann, M. 1999. Quantitative proteomics [news]. *Nat.Biotechnol.* 17:954 - 955.

가

IV.

(KHUPO)

[\(www.hupo.org/aohupo/\)](http://www.hupo.org/aohupo/)

가

【 】

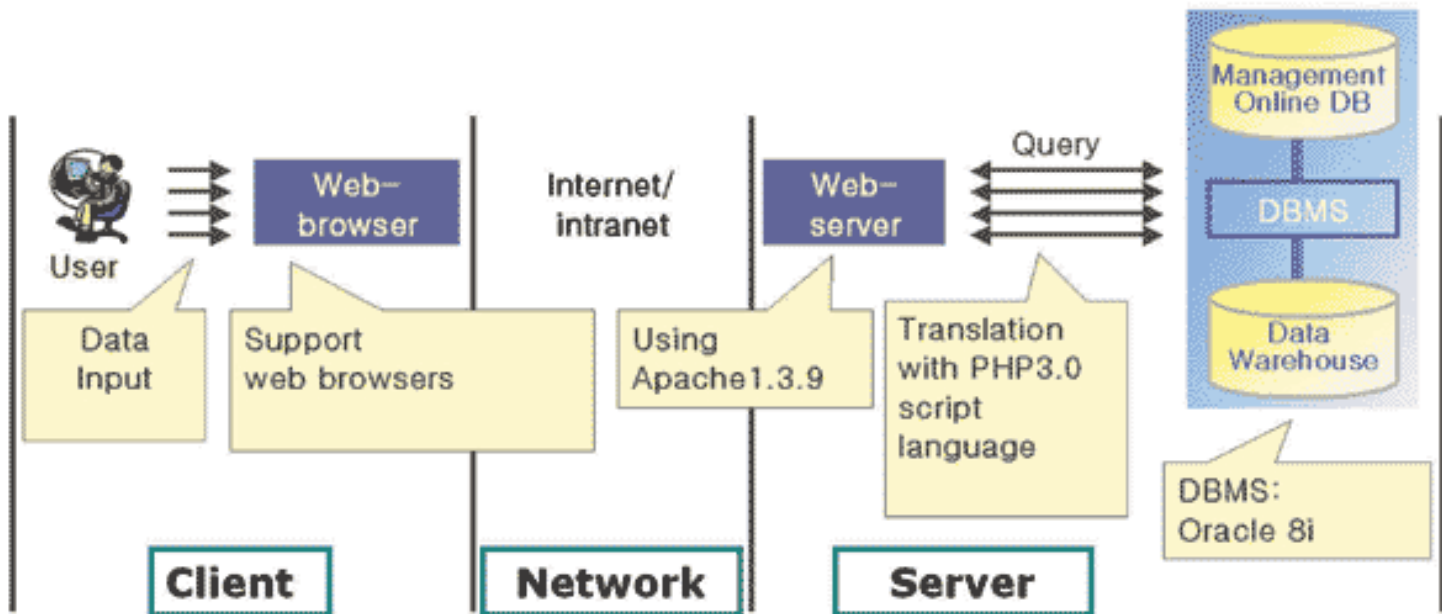
1. Wilkins, M. R., K. L. Williams, R. D. Appel, and D. F. Hochstrasser (eds.), Proteome Research: New Frontiers in Functional Genomics. Springer, Berlin.
2. Blackstock, W. P. and M. P. Weir. 1999. Proteomics: quantitative and physical mapping of cellular proteins. Trends Biotechnol. 17:121 - 127.
3. Park KS, Cho SY, Kim H, Paik YK. Proteomic alterations of the variants of human aldehyde dehydrogenase isozymes correlate with hepatocellular carcinoma. Int J Cancer. 2002 Jan 10;97(2):261 - 5.

12. Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R. Quantitative analysis of complex protein mixtures using isotope - coded affinity tags. Nat Biotechnol. 1999 Oct;17(10):994 - 9.
13. Gerard Cagney & Andrew Emili, 2002, de novo peptide sequencing and quantitative profiling of complex protein mixtures using mass - coded abundance tagging, Nat Biotech.,20,163 - 170



HUPO(Human Proteome Organization)

1975	()
1983	Univ. Missouri - Columbia ()
1983 -	Univ. California, San
1986	Francisco (Post. Doc)
1986 -	Gladstone Institute of
1989	Cardiovascular Diseases, Univ. California
1989 -	
1993	
1993 -	
1994	
1994 -	
2001	HUPO(Human Proteome Organization)
2002	HUPO(Human Proteome Organization)



❖ Select PHP 3.0 script language for whole platform on OS, relational DBMS

7 DB (YPRC - PDB)

1 2 3 4 5 6 ()

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