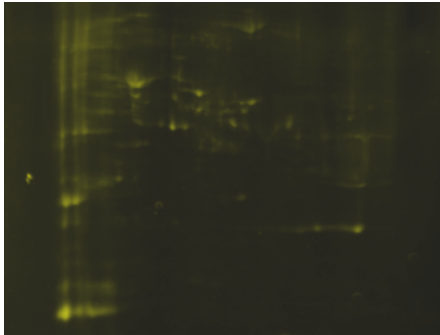


Service item1 Phosphoprotein staining service

Cat. No. P8010



Yeast cell lysate ProQ staining

Step1

Proteome : total cell lysate

Protein separation : 2-DE mini gel format

1st Dimension IEF : 4-10 NL IPG(8.5cm)

2nd Dimension slab gel : 8.5 X 8cm slab gel

Staining method :

staining 1 : ProQ Diamond staining (Molecular Probes™)

staining 2 : Colloidal CBB staining

Detection method : ProQ fluorescent emission (Ex/Em: 555/580nm)

used filter : Ex/Em: Cy3/Cy3

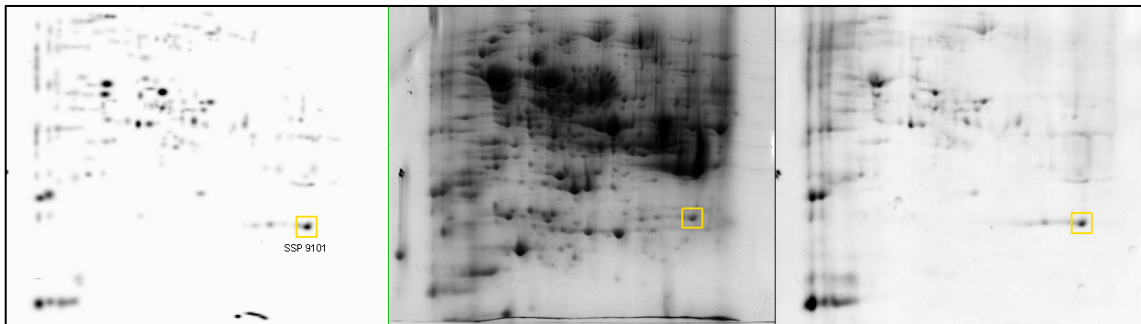
Step2

Multiple staining & spot matching

Synthetic image

All protein staining (CBB)

Phosphoprotein staining (ProQ)



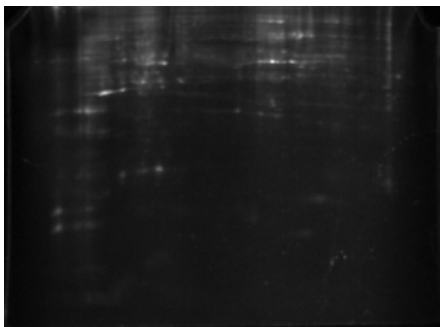
Yeast cell lysate

IEF : 400ug protein from yeast lysate

Detection ProQ Diamond staining & CBB

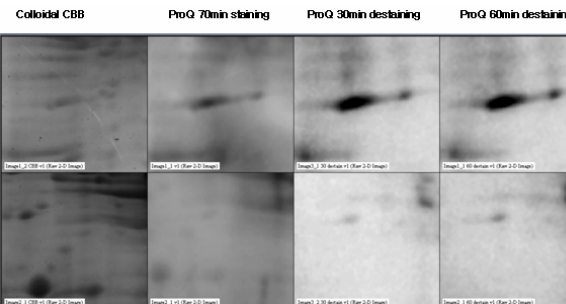
Step3

Differential display & Comparative analysis



DAE3

DAE19



Arabidopsis seedling

IEF : 300ug protein from Arabidopsis lysate

Detection ProQ Diamond staining & CBB

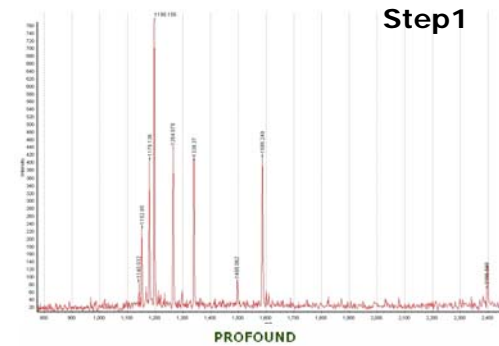
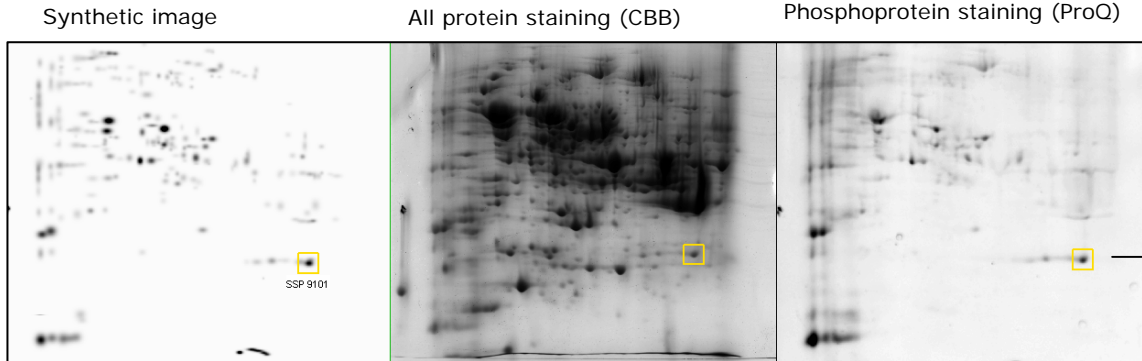
Service item2 Phosphoprotein identification by PMF

Cat. No. P8020

This service give rise to protein identification and/or candidates of possible phosphorylated peptides

Step1 : Protein identification by peptide mass fingerprinting

Step2 : Prediction of possible phosphorylated peptide by peptide mass matching for phosphorylation modification



Protein Candidates

Rank	Probability	Est'd Z	Protein Information and Sequence Analyse Tools (T)	%	PI	kDa	R
+1	1.0e+000	1.99	gi6322583 ref NP_012657.1 Protein component of the small (40S) ribosomal subunit, the least basic of the non-acidic ribosomal proteins; phosphorylated in vivo; essential for viability; has similarity to E. coli S7 and rat S5 ribosomal proteins [Saccharomyces cerevisiae]	27	8.6	25.08	⊖

Step2

ProFound - Search Result Details

Details for rank 1 candidate in search A7BE074B-11AC-B12A170E
1. [gi6322583|ref|NP_012657.1](#) Protein component of the small (40S) ribosomal subunit, the least basic of the non-acidic ribosomal proteins; phosphorylated in vivo; essential for viability; has similarity to E. coli S7 and rat S5 ribosomal proteins [Saccharomyces cerevisiae]
2. [gi1173269|ref|P26783](#) R55_YEAST 40S ribosomal protein S5 (S2) (Y5B) (RP14)
3. [gi89589|emb|CAA61550.1](#) ribosomal protein S5 [Saccharomyces cerevisiae]
4. [gi1013849|emb|CAA89654.1](#) RP55 [Saccharomyces cerevisiae]
5. [gi1013187|tbl|AA192887.1](#) YJR123W [Saccharomyces cerevisiae]

Sample ID : [Para:0]
Measured peptides : 9
Matched peptides : 7
Min. sequence coverage: 27%



Measured	Seq	Coverage	Error	Passive	Missed		
Resid	Score	Start	End	Start	End		
1139.024	M	1139.641	-0.617	187	166	1	EQAVV99PLS
1139.024	M	1139.641	-0.617	188	167	1	QAVV99PLRS
1451.042	M	1451.945	-0.509	92	102	0	LRVLDKDDG
1151.042	M	1151.549	-0.507	46	54	0	WRPFRVSR
1139.148	M	1139.646	-0.497	66	76	0	QPFVAVTAGR
1263.973	M	1263.446	-0.874	85	65	0	DAKLVV9VGR
1339.262	M	1339.798	-0.436	148	100	0	VQDAKALITGQR
1474.054	M	1474.099	-0.945	167	180	1	IQVDAKALITGQR
1685.241	M	1685.055	-0.614	189	203	0	TRARLAEKLR

Spot ID	Protein annotation	MW/pI	Predicted phosphorylation site
9101	Protein component of the small (40S) ribosomal subunit	25.08/8.6	LTNSLMMNGR (2)+HPO3@STY;
0103	acetolactate synthase (EC 4.1.3.18) regulatory chain	34.31/6.1	GFNIDSLVVCNTEVKDLRS (1)+HPO3@STY; VVASSCATMVRCSSTSALAYK (4)+HPO3@STY
1101	elongation factor-1 beta	22.67/4.3	
2202	BMH1	22.13/5.9	
7304	Met17p	48.71/6.0	TKAVYLETIGNPK (3)+HPO3@STY
6505	Glk1p	55.76/5.8	GLPMIPAFVTGSPNGTER (1)+HPO3@STY
4302	glycerol-3-phosphate dehydrogenase	43.42/7.5	LIKDPTSGK (2)+HPO3@STY STKMIHGVRR (1)+HPO3@STY TLEPIPK (1)+HPO3@STY EAVLVTPTNFSEEQK (2)+HPO3@STY
3601	Hsp70 protein	58.33/4.9	
3804	ubiquitin-activating enzyme	114.85	
3806	Silent information regulator	114.85	IDHTIAWAK (1)+HPO3@STY LTESYSSRRDPPEK (2)+HPO3@STY
6805	Elongation factor 3A (EF-3A) (EF-3)	116.83/5.7	LSVATADNR (2)+HPO3@STY
5401	Tif3p	48.5/5.2	GSNFOSSRRPERR (1)+HPO3@STY
4501	Hexokinase isoenzyme 2 that catalyzes phosphorylation	54.15/5.2	HFISELEKGLSK (2)+HPO3@STY
8101	Protein component of the small (40S) ribosomal subunit	25.08/8.6	
1002	HFA1	92.83/6.3	LGELGVYSAGTVEVLYSPK (1)+HPO3@STY
0102	GTP binding protein	24.96/8.6	RHLTGFEEK (1)+HPO3@STY
6101	Asc1p	34.90/5.8	LWDVATGETYQRFVGHK (3)+HPO3@STY

Service item3 Phosphopeptide identification by PSD

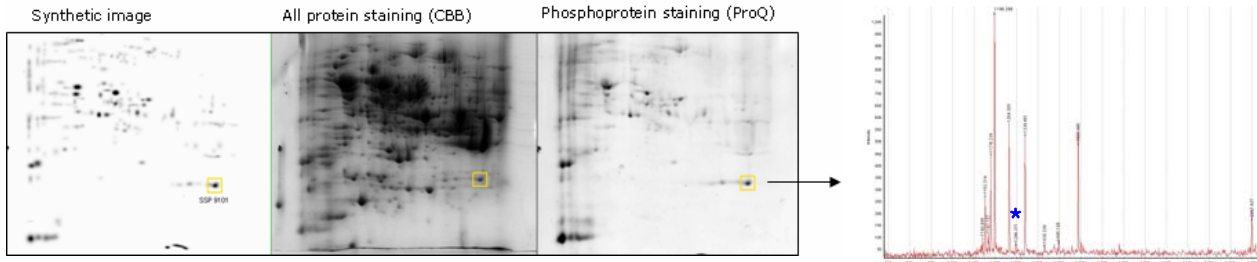
Cat. No. P8030

Alternative Step1: Prediction of Phosphopeptides by PMF

Alternative Step2(example) : Phosphopeptide enrichment from ProQ-stained spots by **Phos-pep™**

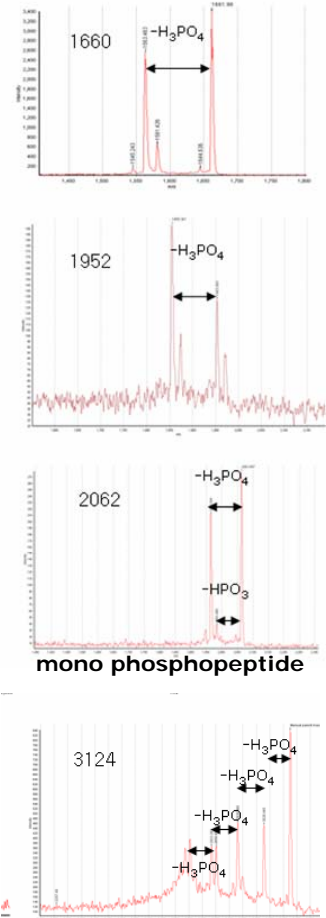
Step3 : Phosphopeptide identification by **MALDI-TOF PSD**

Alternative Step1(example)



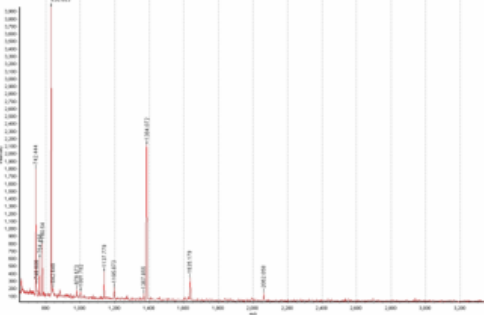
Spot ID	Protein annotation	MW/pI	Predicted phosphorylation site
9101	Protein component of the small (40S) ribosomal subu	25.08/8.6	LTNSLMMNGR (1)+HPO3@STY;

Step3

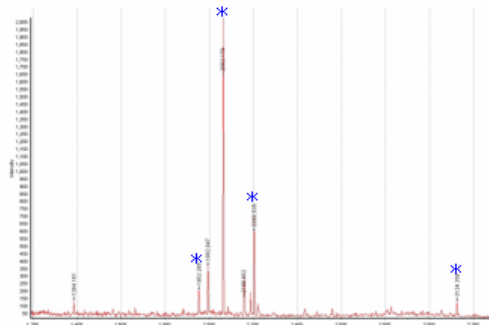


Alternative Step2(example)

Trypsin digest of beta-casein

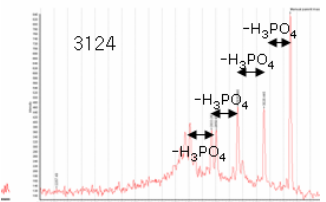


Phos-pep™ Non-IMAC enrichment



Note : In step3, phosphopeptides could be identified by detecting loss of phosphorous(-80Da) or phosphate(-98Da) group in mass spectrometry with PSD mode

Tetra phosphopeptide



Service item4 Phosphorylation site determination by CAF sequencing

Cat. No. P8040

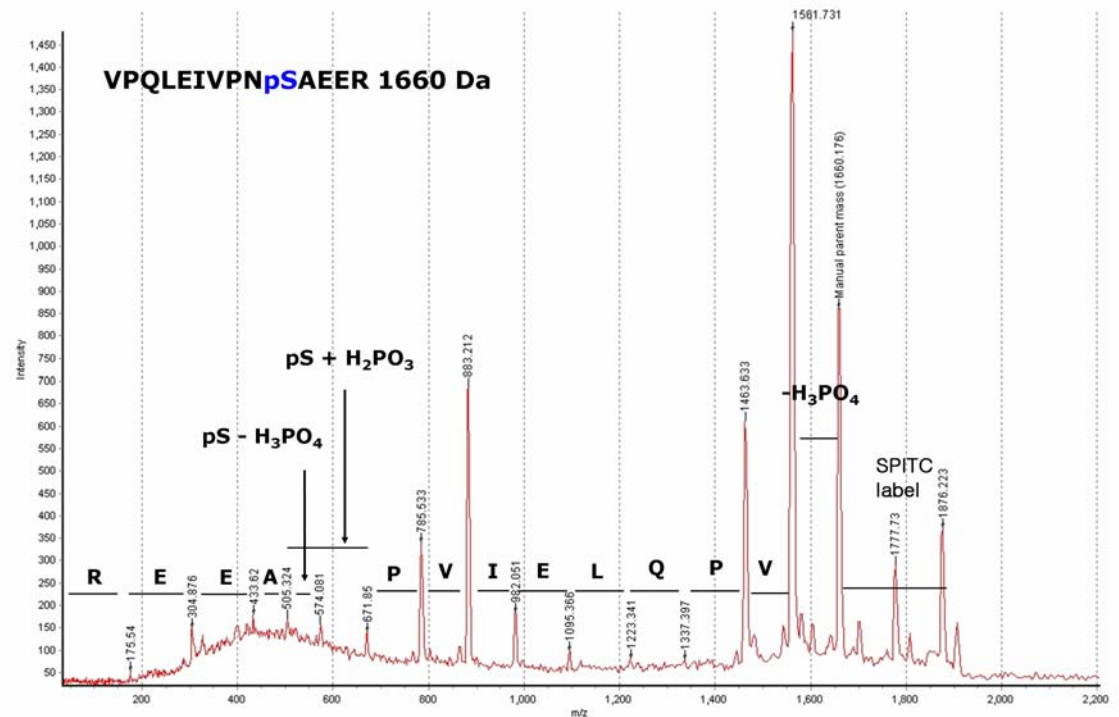
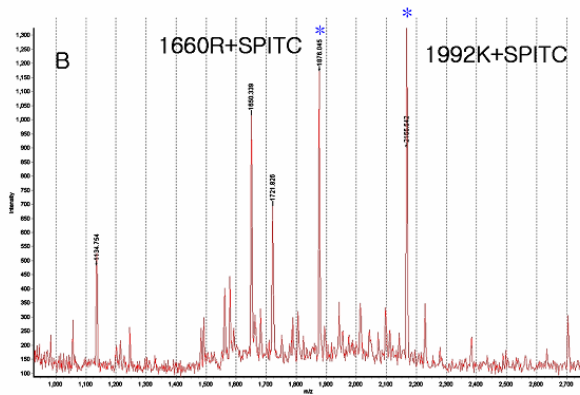
Step1 : SPITC labeling of Trypsin digest from ProQ-stained spot

Step2 : phosphopeptide Enrichment by Phos-pep™

Step2 : CAF-sequencing and phosphorylation site determination by MALDI-TOF PSD

Step1 & 2

Step3



Service item5 Phosphoprotein enrichment plus Service item 1

Cat. No. P8050

This service provide more chance to meet low abundant phosphorylated proteins by phosphoprotein-specific enrichment from total protein lysate.

Step1 : Phosphoprotein enrichment from total cell or tissue lysate by Phos-Pro™ kit(Genomine's Proprietary Technology)

Step2 : Phosphoprotein staining service (service item1)

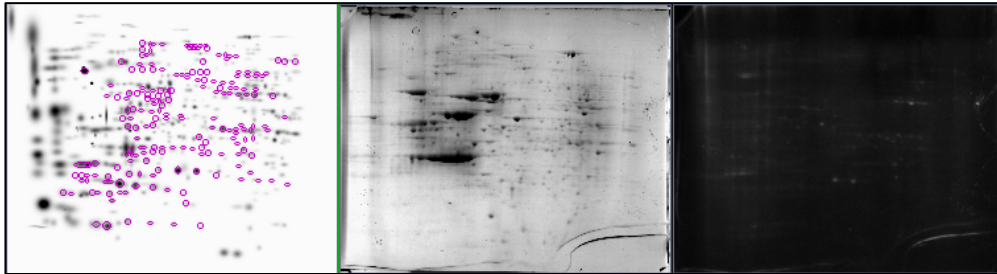
Synthetic image

h460 total protein
(CBB staining)

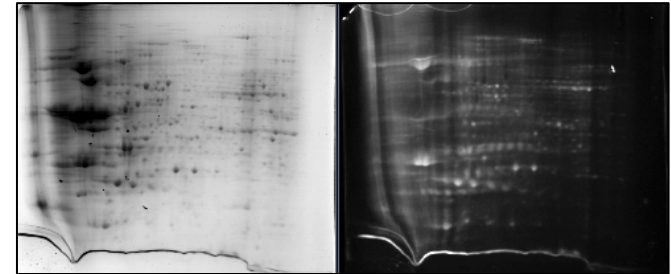
h460 total protein
(ProQ staining)

h460 Phosphoprotein
enriched (CBB staining)

h460 Phosphoprotein
enriched (ProQ staining)



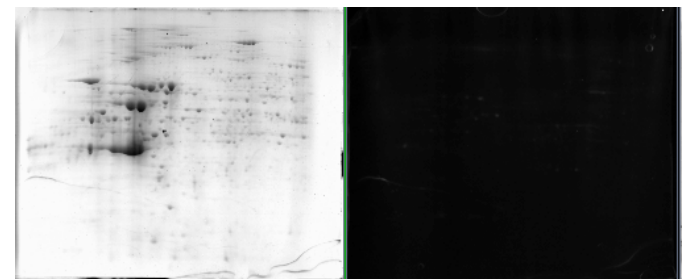
Loading sample : 300ug h460 total cell lysate
Phosphoprotein staining(ProQ) : 35 protein spots was stained as phosphoproteins



Loading sample : 300ug enriched proteins from 6mg
h460 total cell lysate
Phosphoprotein staining(ProQ) : 198 protein spots was stained as phosphoproteins

h460 residual supernatant
fraction (CBB staining)

h460 residual supernatant
fraction (ProQ staining)



Loading sample : 300ug residual protein fraction from
enrichment. Few protein spots was detected as
phosphoproteins